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

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

CONVERSION OF SCIENTIFIC NOTATION

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

EXECUTIVE SUMMARY

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

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

13
14 The U.S. EPA has developed a registration review program for pesticides which operates on a
15 15-year cycle. Spinosad is currently under registration review which is scheduled for completion
16 in 2017 (U.S. EPA/OPP 2012a, p. 8). While the final risk assessments on spinosad from the
17 registration review will not be available during the conduct of the current Forest Service risk
18 assessment, several relevant documents in support of the registration review have been released
19 by EPA and are used in the preparation of the current risk assessment (U.S. EPA/OPP
20 2011b,2012a; U.S. EPA/OPP/HED 2011a (registration review scoping), U.S. EPA/OPP/HED
21 2011b (human incidents); U.S. EPA/OPP/HED 2012a (response to public comment); U.S.
22 EPA/OPP/EFED 2011a (preliminary assessment) U.S. EPA/OPP/EFED 2012a (response to
23 public comments); U.S. EPA/OPP/EFED 2009b; U.S. EPA/OPP/EFED 2011a).

24
25 In addition to the documents from EPA, additional risk assessments are available from USDA's
26 Animal and Plant health protection service (USDA/APHIS 1999, 2003, 2011, 2014) as cited
27 above, the European Food Safety Authority (EFSA 2011, 2012, 2013, 2014), the World Health
28 Organization (FAO/WHO 2001; WHO 2008, 2011), and reviews and assessments in the open
29 literature (Biondi et al. 2012; Cleveland et al. 2002a,b; Dow 2014; Dow Elanco 1996; Elanco
30 2012; Gao et al. 2007b; HSDB 2003 [Spinosyn-A only]; Kirst et al. 1992; Mandal et al. 2013;
31 Mayes et al. 2003; McCormack 2011; McFadden and Saunders 2004; Miles and Eelen 2006;
32 Sparks et al. 1998; Thompson et al. 2015; Williams et al. 2003b). For the most part, reviews of
33 spinosad are used primarily to identify key studies from the open literature and not as direct
34 sources of information. Exceptions to this approach are discussed in the body of this risk
35 assessment as appropriate.

36 **1.2. General Information**

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

43
44 This is a technical support document which addresses some specialized technical areas.
45 Nevertheless an effort was made to ensure that the document can be understood by individuals
46 who do not have specialized training in the chemical and biological sciences. Certain technical

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

7
8 As noted in Section 1.1, the studies submitted in support of the registration of spinosad are used
9 extensively in this risk assessment based on information publically available from the U.S. EPA.
10 In any risk assessment based substantially on registrant-submitted studies, the Forest Service is
11 sensitive to concerns from members of the general public of potential bias. The general concern
12 might be expressed as follows:

13
14 *If the study is paid for and/or conducted by the registrant, the study may*
15 *be designed and/or conducted and/or reported in a manner that will*
16 *obscure any adverse effects that the compound may have.*
17

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

38
39 While data quality and data integrity are not substantial concerns, a limitation in risk assessments
40 based substantially on registrant-submitted studies involves the nature and diversity of the
41 available studies. The studies required by the U.S. EPA are based on a relatively narrow set of
42 criteria in a relatively small subset of species and follow standardized protocols. The relevance
43 of this limitation to the current risk assessment on spinosad is noted in various parts of this risk
44 assessment as appropriate. As discussed in Section 1.1, the open literature on spinosad is
45 focused on efficacy studies but includes studies relevant to the assessment of both potential
46 human health effects as well as effects on terrestrial and aquatic nontarget species. The open

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

4
5 The Forest Service periodically updates pesticide risk assessments and welcomes input from the
6 general public and other interested parties on the selection of studies included in risk
7 assessments. This input is helpful, however, only if recommendations for including additional
8 studies specify why and/or how the new or not previously included information would be likely
9 to alter the conclusions reached in the risk assessments.

10
11 As with all Forest Service risk assessments, almost no risk estimates presented in this document
12 are given as single numbers. Usually, risk is expressed as a central estimate and a range, which
13 is sometimes quite large. Because of the need to encompass many different types of exposure as
14 well as the need to express the uncertainties in the assessment, this risk assessment involves
15 numerous calculations, most of which are relatively simple. Simple calculations are included in
16 the body of the document [typically in brackets]. The results of some calculations within
17 brackets may contain an inordinate number of significant figures in the interest of
18 transparency—i.e., to allow readers to reproduce and check the calculations. In all cases, these
19 numbers are not used directly but are rounded to the number of significant figures (typically two
20 or three) that can be justified by the data.

21
22 Notwithstanding the above, some of the calculations used in this risk assessment are
23 cumbersome. For those calculations, EXCEL workbooks (i.e., sets of EXCEL worksheets) are
24 included as attachments to this risk assessment. The workbooks included with the current risk
25 assessment are discussed in Section 2.4. The worksheets in these workbooks provide the detail
26 for the estimates cited in the body of the document. Documentation for the use of these
27 workbooks is presented in SERA (2011a).

28
29 The EXCEL workbooks are integral parts of the risk assessment. The worksheets contained in
30 these workbooks are designed to isolate the numerous calculations from the risk assessment
31 narrative. In general, all calculations of exposure scenarios and quantitative risk
32 characterizations are derived and contained in the worksheets.

33
34 In the EXCEL worksheets as well as in the text of this risk assessment, the hazard quotient (HQ)
35 is used to characterize risk. The HQ is the ratio of the estimated exposure to a toxicity value,
36 typically a no adverse effect level or concentration (e.g. RfD, NOAEL or NOAEC). Both the
37 rationale for the calculations and the interpretation of the hazard quotients are contained in this
38 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

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

5
6 The chemical and physical properties of spinosad are summarized in Table 2. Spinosad,
7 particularly spinosyn A, has a high affinity for soils with most K_{oc} values greater than 1000.
8 While both spinosyns A and D are highly lipophilic (i.e., high K_{ow} values), these compounds do
9 not tend to bioconcentrate substantially in fish (BCFs below 100). Both spinosyn A and
10 spinosyn D have relatively short half-lives in soil (<20 days) but are metabolized in the
11 environment to compounds that are very similar to the parent compounds (U.S. EPA/OPP/EFED
12 2011a). Minor metabolic pathways (i.e., demethylation) have also been noted in mammals
13 (FAO/WHO 2011). Consequently, as discussed further in Section 3.2.3.4 (Contaminated Water),
14 the modeled water concentrations of spinosad assume that spinosad is essentially stable, which is
15 identical to the approach used by EPA in drinking water assessments (e.g., U.S. EPA/OPP/EFED
16 2009b).

17
18 The ratio of spinosyn A to spinosyn D in technical grade spinosad appears to be highly variable.
19 The U.S. EPA (e.g., U.S. EPA/OPP/EFED 2011a, p. 11) and WHO (2008) indicate that the ratio
20 of spinosyns A:D may vary from 50:50 to 95:5 and that a typical ratio is 85:15 (i.e., equivalent to
21 17:3 or about 5.7:1). The variability in the ratios of spinosyns A:D does not appear to be a
22 significant source of uncertainty in the current risk assessment. As discussed further in
23 Section 3.1.5, subchronic bioassays of spinosad with spinosyn A:D ratios of 1:1 and 5:1 appear
24 to have similar toxicities in mammals. Similar studies on receptors of interest to the ecological
25 risk assessment, however, have not been identified.

26
27 Spinosad is closely related to spinetoram, a chemically modified mixture of spinosyns J and L
28 (Dow 2014b). Several EPA human health risk assessments jointly consider spinosyn and
29 spinetoram (e.g., U.S. EPA/OPP 2009a, U.S. EPA/OPP 2011a). Human health risk assessments
30 conducted by the EPA typically consider spinosad and spinetoram as toxicologically equivalent
31 (e.g., U.S. EPA/OPP/HED 2009a, 2011a). As discussed further in Section 3.4, the chronic RfD
32 for spinosad is based on a study with spinetoram. As discussed in Section 4.1, this toxicological
33 equivalence does not hold for the ecological risk assessment, and spinosad is more toxic to
34 terrestrial invertebrates but less toxic to aquatic invertebrates than spinetoram (e.g., U.S.
35 EPA/OPP 2012a, p. 4). The current Forest Service risk assessment is concerned primarily with
36 spinosad. Data on spinetoram are not considered except as necessary to discuss toxicity values
37 used in the current Forest Service risk assessment and EPA risk assessments.

38
39 Spinosad is often referenced in the literature as a “biorational” pesticide (e.g., Jiang and Mulla
40 2009; Marina et al. 2012; Nowak et al. 2001). The term *biorational pesticide* is generally used to
41 designate pesticides that involve low application rates and few nontarget effects (Hall and Barry
42 1995; Horowitz et al. 2009). Consistent with the use of this term in the open literature, the U.S.
43 EPA/OPP (2015a) designates spinosad as a “reduced risk” pesticide—i.e., a pesticide that
44 generally poses fewer risks to humans and other nontarget organisms relative to conventional
45 pesticides.

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

6
7 While spinosad was discovered in the early 1980s (Thompson et al. 2000; Tomlin 2004), it was
8 not registered as a pesticide in the United States until 1997 (U.S. EPA/OPP 2012a). Spinosad
9 was originally registered by DowElanco (now Dow AgroSciences) (Thompson 2015; Tomlin
10 2004; U.S. EPA/OPP/HED 1997) but appears to be off patent with 76 active formulations from
11 several different companies available in the United States (Kegley et al. 2014).

12
13 Spinosad is a broad spectrum pesticide registered for the control of many insect populations
14 (e.g., lepidopteran larvae, flies, thrips and beetles) on various agricultural crops and
15 nonagricultural sites, including tree farms (Dow 2014a; Harrell and Stepanek 2005; Semiz et al.
16 2006; U.S. EPA/OPP 2012a; Wanner et al. 2002). The U.S. EPA granted an emergency
17 exemption to the state of Michigan on June 18, 2010 for the control of Emerald Ash Borer on
18 wood lots

19 (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.
20 2007). Some formulations of spinosad (e.g., Conserve SC and Entrust) are now specifically
21 labelled for the control of the emerald ash borer. These and other formulations of spinosad
22 labelled for forestry use are discussed further below.

23
24
25 The Forest Service has indicated that spinosad may be used for the control of coneworms
26 (*Dioryctria* species) and seed bugs (*Leptoglossus corculus*) in a loblolly pine seed orchards
27 (Mangini 2016) as well as the Nantucket pine tip moth (Nowak et al. 2010). In addition, the
28 Forest Service is considering the use of spinosad to control minor infestations of pine sawflies
29 and other defoliators in and around recreation areas, district offices, work centers and other areas
30 where conventional agricultural pesticides would not be appropriate.

31
32 Representative formulations of spinosad labelled for forestry are given in Table 3. The
33 representative formulations include a dispersible granule (Blackhawk), a wettable powder
34 (Entrust), and three suspension concentrates (Conserve SC, Entrust SC, and SpinTor 2SC).
35 Forestry applications of Conserve are documented in the literature for the control of the Douglas-
36 fir tussock moth, *Orgyia pseudotsugata* (Cranshaw et al. 2014) and several other insect pests on
37 conifers (Nebraska Forest Service 2009). The use of Spin Tor 2SC is documented in the
38 literature for the control of the Nantucket pine tip moth, *Rhyacionia frustrana* (Nowak et al.
39 2000). Spinosad is also used to protect fruit orchards from various insect pests (e.g., Peusens and
40 Belian 2012).

41
42 The list of formulations in Table 3 is not intended to be exclusive. Other formulations of
43 spinosad are available commercially and new formulations of spinosad may become available in
44 the future. The Forest Service may elect to use registered formulations of spinosad relevant to
45 forestry applications other than those summarized in Table 3. If other formulations are used in
46 Forest Service programs, however, attempts should be made to identify information on the inerts

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

3
4 Some information on mammalian toxicity as well as toxicity to nontarget organisms is typically
5 given on MSDSs or SDSs for the formulations. Information on mammalian toxicity from the
6 MSDSs/SDSs is summarized in Table 4. Note that the six types of studies summarized in Table
7 4 – i.e., acute oral, dermal, and inhalation as well as dermal irritation, eye irritation, and skin
8 sensitization -- are sometimes referred to as the “mammalian six-pack”. These types of studies
9 are typically required by the EPA on all unique formulations (NAS 2013). Information on
10 ecological effects from the MSDSs/SDSs is summarized in Table 5. These types of studies are
11 typically required by EPA on the active ingredient but some of these studies may be conducted
12 on formulations.

13
14 If information on mammalian and ecological receptor toxicity from MSDSs/SDSs for another
15 formulation is comparable to the information given in Tables 4 and 5, the other formulation
16 would be encompassed by the current risk assessment unless additional information (e.g., new
17 literature or case reports on the formulation) suggest that the other formulation may be more
18 hazardous than the representative formulations specified in Table 3. The data in Tables 4 and 5
19 are discussed in subsequent sections of this risk assessment as appropriate.

20 **2.3. Application Methods**

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

29
30 As discussed in Section 1.1, this risk assessment is accompanied by EXCEL workbooks that
31 detail the exposure scenarios for spinosad. Based on the anticipated uses of spinosad in Forest
32 Service programs, two EXCEL workbooks are provided, one for a single application
33 (Attachment 1) and the other for multiple applications of spinosad (Attachment 2). The specific
34 application rates and intervals are discussed further in the following section.

35
36 Different application methods involve different estimates of the amount of a pesticide used by
37 workers in a single day based on the number of acres treated per day and the application rate.
38 Application rates are discussed in Section 2.4, and assumptions about the number of acres treated
39 by a worker in a single day are discussed further in Section 3.2.2 (worker exposure assessments).

40 **2.4. Mixing and Application Rates**

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

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

3
4 As summarized in Table 3, the maximum seasonal application rate for the representative forestry
5 formulations of spinosad is 0.45 lb a.i./acre. Oddly, the product label for Conserve SC seems
6 somewhat ambiguous. Like other labels for forestry formulations, the product label for Conserve
7 SC indicates that the maximum seasonal application rate is 0.45 lb a.i./acre; however, it also
8 indicates that up to 88 fluid ounces of the formulation may be applied per acre for the control of
9 some tree pests such as the emerald ash borer. For this 1 lb a.i./gallon formulation, 88 fluid
10 ounces/acre corresponds to an application rate of about 0.69 lb a.i./acre, which exceeds the
11 maximum labelled seasonal application rate of 0.45 lb a.i./acre which appears to apply to forestry
12 applications. For some non-forestry applications, the most recent human health risk assessment
13 from EPA does consider application rates of up to about 0.76 lb a.i./acre (U.S. EPA/OPP/HED
14 2011a, p. 48).

15
16 For the current Forest Service risk assessment, the maximum single application rate (detailed in
17 Attachment 1) is taken as 0.225 lb a.i./acre from the forestry application by Lewis et al. (2009).
18 This study was a joint effort by APHIS, the University of Michigan, and the USDA Forest
19 Service. For multiple applications (Attachment 2), two applications of 0.225 lb a.i./acre with an
20 application interval of 6 days is used. This two-application scenario equals the maximum
21 seasonal application rate of 0.45 lb a.i./acre using the minimum application interval for trees
22 specified on the forestry labels. The maximum seasonal application rate of 0.45 lb a.i./acre is
23 consistent with recent risk assessments from EPA (U.S. EPA/OPP/HED 2009a, p. 13).

24
25 Application volumes, meaning the number of gallons of pesticide solution applied per acre, have
26 an impact on the estimates of potential risk. The extent to which a formulation is diluted prior to
27 application primarily influences dermal and direct spray exposure scenarios, both of which
28 depend on 'field dilution' (i.e., the concentration of spinosad in the applied spray). In all cases,
29 higher herbicide concentrations (i.e., equivalent to the lower dilution of the herbicide) increase
30 the estimate of exposure and hence risk. As summarized in Table 3, minimum application
31 volumes of 5 to 10 gallons per acre are recommended. In the workbooks that accompany this
32 risk assessment, the application volumes are taken as 10 (5 to 20) gallons per acre.

33
34 The selection of specific application rates and dilution volumes in this risk assessment is
35 intended to reflect plausible estimates of potential exposures. In the assessment of specific
36 program activities, the application rates and volumes can be changed in Worksheet A01 of the
37 EXCEL workbooks to reflect the rates and volumes that are actually used in any specific
38 application of spinosad.

39 **2.5. Use Statistics**

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

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

19
20 Detailed pesticide use statistics are compiled by the state of California. The use statistics from
21 California for 2013, the most recent year for which statistics are available, indicate a total use of
22 spinosad of 34,771.95 lbs (CDPR 2015, pp. 691-695). No explicit forestry applications are noted
23 in the California report. The use most closely related to forestry involved rights-of-way
24 applications which consisted of a total of 2.68 pounds (i.e., about 0.0077% of total use). The
25 only other use that might be relevant to Forest Service programs involved applications for
26 landscape maintenance—i.e., 1453.07 lbs or about 4.2% of total use. Public health applications
27 accounted for 6433.09 pounds or about 18.5% of total use. The relevance of this use to the
28 Forest Service appears to be marginal since the Forest Service (Section 2.2) has not indicated
29 that spinosad will be used in public health applications (e.g., mosquito control).

30
31 As with the USGS use statistics, CDPR notes that spinetoram use appears to be displacing
32 spinosad:

33
34 *Spinosad and spinetoram are primarily used in citrus to manage citrus thrips.*
35 *Both are very selective, allowing natural enemies to survive. They may*
36 *eventually erode the market share of older insecticides. Of the two, spinetoram is*
37 *more effective against citrus thrips populations that have developed resistance to*
38 *carbamate insecticides, and its persistence and effectiveness has resulted in the*
39 *reduced use of spinosad. The area treated with spinosad decreased 55 percent in*
40 *2013, while spinetoram use increased 32 percent.*

41 CDPR 2015, p. 114

42
43 Based on the use statistics from California, agricultural uses of spinosad would appear to be
44 much greater than uses related to forestry or other non-agricultural applications. Because
45 spinosad has not been used extensively in Forest Service programs or projects, however, it is

- 1 unclear at this time if Forest Service applications of spinosad would be negligible relative to
- 2 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).

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

9
10 There is some evidence that spinosad may enhance the neurotoxic effects of ivermectin by
11 inhibiting P-glycoprotein transport (Section 3.1.16). P-glycoprotein is an ATP-dependent efflux
12 pump involved in inhibiting the uptake and active secretion of xenobiotics from cells (Ambudkar
13 et al. 2003). While this mechanism may be important in some drug interactions involving
14 spinosad, the role of P-glycoprotein inhibition in the direct toxicity of spinosad is unclear.

15 **3.1.3. Pharmacokinetics and Metabolism**

16 **3.1.3.1. Distribution and Metabolism**

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

27
28 As would be expected of relatively lipophilic compounds, the spinosyns were primarily
29 distributed to fat with substantial amounts also noted in kidneys, lymph nodes, and the thyroid.
30 The open literature study by Rothwell et al. (2005) also notes substantial accumulation of
31 spinosad in the fat of sheep. As discussed further in Section 3.1.5, the thyroid appears to be a
32 target tissue following longer-term exposures to spinosad. As illustrated in Figure 4, spinosyns
33 A and D undergo limited metabolism consisting of N-demethylation or O-demethylation as well
34 as conjugation with glutathione. As discussed further in Section 4.1.2.2, N-demethylation and
35 O-demethylation also appear to be common metabolic processes in birds (Magnussen et al.
36 1996).

37
38 While information on the toxicity of spinosad metabolites to mammals is not available, the *in*
39 *vivo* metabolites as well as environmental metabolites (Section 3.1.15.1) are similar to the parent
40 compound, and the EPA assumes that the metabolites are similar in toxicity to the parent
41 compounds—i.e., spinosyns A and D. As discussed further in Section 4.1.3.3 (hazard
42 identification for aquatic invertebrates), the limited toxicity data on the metabolites of spinosad
43 suggest that the metabolites are comparable in toxicity to the parent compounds (U.S.
44 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} [$\ln(1-0.1) \div 8 \text{ hours} \approx 0.01317 \text{ hour}^{-1}$].

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.

1
2 While the EPA risk assessments do not discuss dermal absorption studies on spinosad, the
3 FAO/WHO (2001) review of spinosad briefly summarizes a dermal absorption study on spinosyn
4 A in rats that was submitted to WHO by Dow AgroSciences, United Kingdom. In this study,
5 cited in FAO/WHO (2001) as Domoradzki and Shabrang 1996, 1% of dermally applied spinosyn
6 A was absorbed by rats over a 24 hour exposure period. Assuming first-order absorption, these
7 results correspond to a first-order dermal absorption rate coefficient of about $0.00042 \text{ hour}^{-1}$
8 $[\ln(1-0.01) \div 24 \text{ hours} \approx 0.0004188 \text{ hour}^{-1}]$.

9
10 Forest Service risk assessments typically consider the use of quantitative structure activity
11 relationships (QSAR), as detailed in SERA (2014a, Section 3.1.3.2.2). The QSAR method is
12 based exclusively on dermal absorption data from studies in humans involving numerous
13 chemicals. As detailed in Worksheet B03b of Attachments 1 and 2, the QSAR methods yield
14 estimated dermal absorption rate coefficients for spinosyn A of about $0.00002 (0.0000007-$
15 $0.0005) \text{ hour}^{-1}$ using a K_{ow} value of 10,000 and a molecular weight of 731.98 (Table 1 with
16 values taken from U.S. EPA/OPP/HED 2011a). While the K_{ow} for spinosyn A is within the
17 range of values on which the algorithm is based—i.e., K_{ow} values ranging from 0.0015 to
18 3,000,000—the molecular weight of spinosyn A exceeds the range of molecular weights on
19 which the algorithm is based—i.e., 60 to 400 g/mole.

20
21 The current Forest Service risk assessment uses the estimated dermal absorption rate coefficients
22 of $0.00002 (0.0000007-0.0005) \text{ hour}^{-1}$ based on the QSAR method from SERA (2014a, Section
23 3.1.3.2.2). While the high molecular weight of spinosyn A diminishes confidence in the
24 estimates from the QSAR algorithm, it should be noted that the upper bound of the estimate does
25 encompass the first-order dermal absorption rate coefficient in rats of about $0.00042 \text{ hour}^{-1}$ from
26 the study by Domoradzki and Shabrang (1996). More significantly, as noted above, the
27 algorithm from SERA (2014a) is based on human data. As reviewed by Ravenzwaay and
28 Leibold (2004), rat skin is more permeable than human skin by about a factor of about 10. Thus,
29 the rate coefficient of $0.00042 \text{ hour}^{-1}$ in rats would suggest a comparable rate coefficient in
30 humans of about $0.00004 \text{ hour}^{-1}$, which is close to the central estimate of $0.00002 \text{ hour}^{-1}$ from the
31 QSAR algorithm.

32
33 While the current Forest Service risk assessment does not adopt the same approach used by
34 EPA—i.e., dermal exposure is negligible—this difference from EPA does not materially impact
35 the current risk assessment. The dermal absorption rate coefficients of $0.00002 (0.0000007-$
36 $0.0005) \text{ hour}^{-1}$ are extremely low. As discussed further in Section 3.4 (risk characterization),
37 none of the dermal exposures for workers or members of the general public approaches a level of
38 concern, which is consistent with the EPA risk characterizations.

39 **3.1.3.2.2. Zero-Order Dermal Absorption**

40 Exposure scenarios involving the assumption of zero-order dermal absorption require an estimate
41 of dermal permeability (K_p) in units of cm/hour. No experimental data are available on the
42 dermal permeability rate of spinosad as a mixture or on spinosyns A or D. In the absence of
43 experimental data, Forest Service risk assessments generally use a QSAR algorithm developed
44 by the EPA (U.S. EPA/ORD 1992, 2007). This approach is discussed in further detail in SERA
45 (2014a, Section 3.1.3.2.1). As with the algorithm for estimating the first-order dermal absorption
46 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 (T_{50}) [$k = \ln(2) \div T_{50}$]. 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 N^{th} dose ($X_{N\ Dose}$) relative to the body burden immediately following the first dose ($X_{1\ Dose}$) is:

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

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 (X_{Inf}) can be calculated as:

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

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} [$\ln(2) \div 1.25 \text{ days} \approx 0.5545 \text{ day}^{-1}$]. 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} [$\ln(2) \div 10 \text{ days} \approx 0.0693 \text{ day}^{-1}$]. 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 [$15 \div 2.4 =$

1 6.25]. As discussed further in Section 3.1.5 (subchronic and chronic toxicity), dogs appear to be
2 more sensitive than rats to spinosad. It seems only modestly speculative to suggest that the
3 greater sensitivity in dogs may be due to the slower elimination of spinosad by dogs relative to
4 rodents.

5
6 As discussed further in Section 3.1.5, the LOAELs for dogs following subchronic and chronic
7 exposures do not differ remarkably – i.e., the subchronic (90 day) LOAEL is about 10.1 mg/kg
8 bw and the chronic (1 year) LOAEL is 8.34 mg/kg bw/day. As also discussed in Goldstein et al.
9 (1974, p. 321), the fractional value of the eventual steady state (f) can be calculated as:

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

10
11
12
13 Based on the above equation and the elimination rate coefficient for dogs of 0.07 day^{-1} , dogs
14 would reach about 0.998 of the eventual plateau by 90 days [$1 - e^{-0.07*90} \approx 0.998164$].
15 Consequently, there would be no substantial difference in body burden for a dog following
16 exposures of 90 days (subchronic exposure) and 1 year (chronic exposure). Thus, the similarities
17 between the subchronic and chronic LOAELs for dogs are consistent with the apparent excretion
18 kinetics of spinosad in dogs.

19 **3.1.4. Acute Oral Toxicity**

20 **3.1.4.1. Standard Registrant Studies**

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

29
30 Acute oral LD_{50} values for spinosad are summarized in Appendix 1, Table A1-1. All of the
31 acute oral toxicity studies appear to involve technical grade spinosad. The EPA classifies
32 spinosad as Category III ($LD_{50} > 500 \text{ mg/kg bw}$, $< 5000 \text{ mg/kg bw}$) for acute oral toxicity based
33 on the acute oral LD_{50} values of $> 2000 \text{ mg a.i./kg bw}$ in rats for technical grade spinosad (U.S.
34 EPA/OPP/HED 2009a, Attachment 2, MRID 00132519).

35
36 A definitive LD_{50} value of 3738 mg/kg bw is reported for male rats. This LD_{50} value is reported
37 in three different sources, each of which provides somewhat different details in terms of
38 experimental design or study attribution. The DER from EPA attributes this study to Gilbert et
39 al. (1994, MRID 43414515) while FAO/WHO (2001) attributes this study to Stebbins and
40 Brooks (1999a). This minor discrepancy probably reflects differences in submissions of the
41 study by the registrant to EPA and WHO. The summary of this study in U.S. EPA/OPP/HED
42 (1997b) notes that the reported LD_{50} of $3738 \text{ mg a.i./kg bw}$ appears to be a combination of the
43 data from two different submissions—i.e., Gilbert et al. 1994 (MRID 43414515) and Wright et
44 al. 1992 (MRIDs 43770701 and 43414515). This supposition appears to be correct in that the

1 study by Gilbert et al. (1994) involved a single dose of 5000 mg a.i./kg bw and the study by
2 Wright et al. (1992) involved a single dose of 5000 mg/kg bw. Thus, the reported definitive
3 LD₅₀ involves zero degrees of freedom.

4
5 The only other definitive LD₅₀ values reported for spinosad are the LD₅₀ values of 6100 mg/kg
6 bw in male mice and 7100 mg/kg bw in female mice. These definitive LD₅₀ values are reported
7 in the FAO/WHO (2001) review and are attributed to a study by Gilbert and Yano (1996). The
8 EPA risk assessments on spinosad report only an indefinite LD₅₀ of >5000 mg/kg bw (Gilbert et
9 al. 1994 MRID 43414515). The study by Gilbert et al. (1994, MRID 43414515) is a limit test
10 that involved only a single dose, which means it could not have been used to estimate the
11 definitive LD₅₀ values reported in FAO/WHO (2001). Given the lack of detail in the FAO/WHO
12 (2001) summary and the limitations in the definitive LD₅₀ for rats (discussed above), the
13 differences in the reported definitive LD₅₀ values in rats and mice cannot be overly interpreted in
14 terms of differences in species sensitivity.

15
16 All of the MSDS for the representative formulations specify acute oral LD₅₀ values of >5000
17 mg/kg bw for rats (Table 4). This toxicity value is consistent with the LD₅₀ for female rats from
18 MRID 43414515 (Appendix 1, Table A1-1) but not with some of the lower LD₅₀ values. The
19 specification of the LD₅₀ of >5000 mg/kg bw/day in mice is consistent with Gilbert et al. (1994,
20 MRID 43414515) as well as Gilbert and Yano (1996 as summarized in FAO/WHO 2001).

21 **3.1.4.2. Other Data**

22 One human poisoning incident is reported in the open literature (Su et al. 2011). The incident
23 occurred in Taipei, Taiwan and was associated with a suicide attempt in which an 80-year old
24 woman consumed both 80 mL of Conserve (11.6% spinosad or about 9 g a.i.) as well as 2 to 3
25 grams of flonicamid. The woman evidenced signs of neurotoxicity within 3 hours of dosing and
26 recovered after prompt medical treatment. Like spinosad, flonicamid is neurotoxic to insects but
27 does not cause signs of neurotoxicity in humans. Flonicamid is somewhat more toxic to rats than
28 spinosad with acute oral LD₅₀ values of 884 mg/kg bw in male rats and 1768 mg/kg bw in female
29 rats (U.S. EPA/OPP/HED 2014a). The body weight of the woman is not specified in the report
30 from Su et al. (2011). Taking 50 kg as an approximate weight of a female from Taiwan (Tao
31 2014), the woman may have consumed a dose of 180 mg/kg bw of spinosad or about 5% of the
32 rat oral LD₅₀ [$180 \text{ mg/kg bw} \div 3738 \text{ mg a.i./kg bw} \approx 4.8154\%$] and a dose of 50 mg/kg bw [2500
33 $\text{mg} \div 50 \text{ kg}$] of flonicamid or about 3% of the LD₅₀ for female rats [$50 \text{ mg/kg bw} \div 1768 \text{ mg/kg}$
34 $\text{bw} \approx 2.82895\%$]. Consistent with the discussion by Su et al. (2011), these dose estimates would
35 not clearly indicate the involvement of either spinosad or flonicamid in the effects on the patient
36 and suggest that other ingredients in the formulation may have contributed to the adverse effects.

37
38 In addition to the poisoning incident in Taiwan, the U.S. EPA/OPP/HED (2011b) reviewed
39 human incidents involving spinosad in the OPP Incident Data System. The EPA review provides
40 few details but does note that six incidents involving spinosad have been reported and that
41 *...most are of lower severity* (U.S. EPA/OPP/HED 2011b, p. 3). Additional details of the effects
42 noted and levels of exposure are not provided.

43
44 Spinosad is used for the treatment of fleas in both cats and dogs. Adverse effects following oral
45 doses in the range of 50 to about 100 mg/kg bw have been associated with vomiting in some
46 studies (Elanco 2012; Paarlberg et al. 2013; Elanco Animal Health 2007). Other studies indicate

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

5 **3.1.5. Subchronic or Chronic Systemic Toxic Effects**

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

11
12 The subchronic and chronic toxicity studies on spinosad are summarized in Appendix 1, Table
13 A1-2. Most of the studies relevant to the current risk assessment were submitted to the U.S.
14 EPA/OPP in support of the registration of spinosad, and the summaries of these studies are taken
15 from EPA human health risk assessments (U.S. EPA/OPP/HED 1997a, 2009a, 2014a). Some
16 repeated dose studies published in the open literature are from the Dow Chemical Company and
17 appear to be identical to studies submitted to EPA, as specified in Appendix 1, Table A1-2 (i.e.,
18 Stebbins et al. 2002; Yano et al. 2002). The subchronic study in rats by El-Hoda et al. (2012)
19 focuses on cellular aberrations in bone marrow, as discussed further in Section 3.1.10
20 (Mutagenicity and Carcinogenicity). The FAO/WHO (2001) review of spinosad provides
21 detailed summaries of several subchronic and chronic studies. As discussed below, some of
22 these studies do not appear to have been submitted to EPA, and some of these studies provide
23 some insight on the similarities of various blends of spinosyns A and D in spinosad.

24
25 Relatively standard studies regarding the subchronic toxicity of spinosad in dogs are available
26 (MRID 43444102), mice (MRID 43566602; Stebbins et al. 2002), and rats (MRID 43566601;
27 Wilmer et al. 1993, MRID 43557504). Standard chronic toxicity studies are also available on
28 these species—i.e., dogs (Harada 1995, MRID 43701504), mice (Bond et al. 1995a, MRID
29 43701505; MRID 44123601), and rats (Bond et al. 1995b, MRIDs 43701507 and 43710503;
30 Spencer and Yano 1995, MRID 43701507 and 43701503).

31
32 As noted in Section 3.1.2 (Mechanism of Action), one of the most common signs of subchronic
33 or chronic exposure to spinosad involves cytoplasmic vacuolation in the cells of many organs,
34 including the thyroid, parathyroid glands, liver, kidney, and stomach. This effect is noted
35 specifically in subchronic and/or chronic studies in mice (MRID 43566602; Bond et al. 1995a,
36 MRID 43701505), rats (MRID 43566601, Bond et al. 1995b, MRIDs 43701507 and 43710503),
37 and dogs (Harada 1995, MRID 43701504).

38
39 In terms of species differences following subchronic or chronic exposures to spinosad, dogs
40 appear to be somewhat more sensitive than rodents. The data on subchronic and chronic
41 NOAELs and LOAELs for mice, rats, and dogs are summarized in Table 6 and illustrated in
42 Figure 5. The additional details of these studies are summarized in Appendix 1 (Table A1-2).
43 For studies that provide separate NOAELs and LOAELs for males and females, the NOAELs
44 and LOAELs given in Table 6 are presented as the arithmetic average of the values for males and
45 females, with all values rounded to the nearest tenth.

1
2 As discussed in Section 2.2, the proportion of spinosyns A and D in spinosad is variable, ranging
3 from about 1:1::A:D to 5.7:1::A:D. The registrant studies summarized in EPA risk assessments
4 do not generally provide information on the ratios of spinosyns A and D in spinosad. The review
5 by FAO/WHO (2001) does summarize the results of two subchronic studies in rats, one study
6 using a 1:1 ratio of spinosyn A to spinosyn D and the other using a 5:1 ratio of spinosyn A to
7 spinosyn D. The study using the 1:1 mixture reports a LOAEL of 39 mg/kg bw/day in males and
8 47 mg/kg bw/day in females. The study using the 5:1 mixture reported a LOAEL of 34 mg/kg
9 bw/day in males and 39 mg/kg bw/day in females. While this is an extremely limited basis for
10 comparison, these studies suggest no substantial differences in the toxicity of spinosad over
11 ranges of spinosyn A to spinosyn D commonly found in commercial formulations.

12
13 In addition to the studies on spinosad, Appendix 1, Table A1-2 also summarizes a subchronic
14 toxicity study on spinetoram in dogs (MRID 47011901). Like spinosad, spinetoram is a mixture
15 of two spinosyns (J and L) which are also fermentation products of *Saccharopolyspora spinosa*.
16 Unlike spinosad, spinosyns J and L are chemically modified in the production of spinetoram
17 (Dow 2014b). Nonetheless, the components in spinetoram are structural analogues to the
18 spinosyns in spinosad. Based on structural similarities and similar toxicological action, spinosad
19 and spinetoram are considered toxicologically equivalent by EPA, at least in terms of human
20 health effects (U.S. EPA/OPP/HED 2009a, 2011a). As discussed further in Section 3.3 (Dose-
21 Response Assessment), the EPA derives the chronic RfD for spinosad based on the chronic study
22 of spinetoram in dogs (MRID 47011901). As summarized in Appendix 1 (Table A1-2), the
23 chronic NOAELs of spinetoram in male dogs (2.96 mg/kg bw/day and female dogs (2.49 mg/kg
24 bw/day) are virtually identical to the chronic NOAELs of spinosad in male dogs (2.66 mg/kg
25 bw/day and female dogs (2.71 mg/kg bw/day). While the current Forest Service risk assessment
26 does not encompass the toxicity studies of spinetoram explicitly, the assessment by U.S.
27 EPA/OPP/HED (2009a, 2011a) on the toxicological equivalence of spinosad and spinetoram
28 seems reasonable. As discussed further in Section 3.3, the current risk assessment defers to EPA
29 on the selection of the spinetoram study as the basis for the chronic RfD for spinosad. As noted
30 above, the NOAELs for spinosad and spinetoram are virtually identical, and the use of the
31 spinetoram study rather than the spinosad study does not materially impact the risk assessment.

32 **3.1.6. Effects on Nervous System**

33 As discussed in Section 4.1.2.4, neurotoxicity is considered the primary endpoint of concern for
34 terrestrial invertebrates. This is not the case for the human health risk assessment. As
35 summarized in Appendix 1 (Tables A1-1 and A1-2), spinosad has been subject to an acute
36 neurotoxicity study (Albee et al. 1994, MRIDs 43557501) and a subchronic neurotoxicity study
37 in rats (Wilmer et al. 1993, MRID 43557504) in rats. In addition, neurotoxicity studies
38 (functional observational batteries) were conducted at months 3, 6, 9, and 12 of the chronic
39 toxicity study in rats (Spencer and Yano 1995, MRID 43701507 and 43701503). The acute and
40 subchronic neurotoxicity studies noted no signs of toxicity (neurotoxic or otherwise) at a dose of
41 2000 mg/kg bw in the acute study and doses of up to 42.7 mg/kg bw/day in males and 52.1
42 mg/kg bw/day in females in the subchronic study. In the chronic study, no signs of neurotoxicity
43 were noted at doses of up to 46.0 mg/kg/day in male and 57.0 mg/kg/day in female rats. Based
44 on these results and consistent with the assessment from U.S. EPA/OPP/HED (2011a, p. 6),
45 neurotoxicity in mammals is not considered an endpoint of concern in the human health risk
46 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,

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

3
4 As summarized in Appendix 1 (Table A1-2), changes in endocrine glands were observed in
5 several subchronic and chronic studies. These changes include vacuolation of the adrenals and
6 pancreas in mice (MRID 43566602), the adrenals in rats (MRID 43566601), and the parathyroid
7 in dogs (Harada 1995, MRID 43701504). Effects on the thyroid include both changes in
8 histopathology in rats (MRID 43566601; Bond et al. 1995b, MRIDs 43701507 and 43710503)
9 and dogs (MRID 47011901, spinetoram only) as well as increased thyroid weights in rats (MRID
10 43566601) and dogs (Harada 1995, MRID 43701504). Increases in thyroid weights were also
11 observed in rats in a reproduction study (Breslin et al. 1994, MRIDs 43701506; Hanley et al.
12 2002), which is discussed further in Section 3.1.9.2. No effects were observed in ovaries or
13 testes.

14
15 As noted in U.S. EPA/OPP/HED (2011a, p. 6), both spinosad and spinetoram are subject to
16 endocrine screening as part of the EPA's Endocrine Disruptor Screening Program. Neither
17 spinosad nor spinetoram have been tested to date, based on information available at the EPA web
18 site for the Endocrine Disruptor Screening Program ([https://www.epa.gov/ingredients-used-
19 pesticide-products/endocrine-disruptor-screening-program-tier-1-assessments](https://www.epa.gov/ingredients-used-pesticide-products/endocrine-disruptor-screening-program-tier-1-assessments)). This status is
20 also noted in the recent USDA/APHIS human health risk assessment on spinosad (USDA/APHIS
21 2014, p. 6). Potential effects on the endocrine system are not addressed in the various spinosad
22 reviews from the European literature (EFSA 2011, 2012, 2013, 2014; European Commission
23 2006; FAO/WHO 2001; WHO 2011).

24
25 In terms of functional effects that have important public health implications, effects on endocrine
26 function could be expressed as diminished reproductive capacity in adults or abnormal fetal
27 development. As discussed in the following section (Section 3.1.9), spinosad does not appear to
28 be associated with specific adverse effects on either fetal development or reproductive
29 performance. Based on these data, the EPA indicated that ... *concern for endocrine-related*
30 *effects is low* (U.S. EPA/OPP/HED 2009a, p. 5). In the absence of mechanistic studies or other
31 clear evidence of disruptions in endocrine function, the current Forest Service risk assessment
32 concurs with the EPA assessments, and effects on endocrine function are not identified as
33 endpoints of primary concern for spinosad.

34
35 One inconsistency in the literature concerns the reproduction study in rats, which is discussed
36 further in Section 3.1.9.1. This study was submitted to the EPA (Breslin et al. 1994, MRIDs
37 43701506) and is published in the open literature (Hanley et al. 2002). The EPA summary of
38 this study indicates that the high dose (100 mg/kg bw/day) resulted in ...*cytoplasmic vacuolation*
39 *of the follicular epithelial cells of the thyroid with increased levels of thyroid-stimulating*
40 *hormone (TSH) and decreased levels of T₄* (U.S. EPA/OPP/2009a, p. 5). The open literature
41 publication provides a summary of the data (Hanley et al. 2002, Table 4) indicating no change in
42 T₄ 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_1) 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).

1 In this study, the parental generations were dosed at 0, 3, 10, or 100 mg/kg bw for 10 weeks (P₁
2 generation) or 12 weeks (P₂ generation). No adverse effects were seen at the two lower doses.
3 At 100 mg/kg bw/day, reduced body weight, increases in relative and absolute weights of heart,
4 kidney, liver, spleen, and thyroid as well as multiple organ pathology (specified in Appendix 1)
5 were observed. In addition, delivery complications resulting in the death of five females were
6 observed at 100 mg/kg bw. Adverse effects in offspring, including smaller litter size/fetal
7 mortality were observed and appeared to be related to maternal toxicity. Based on this study,
8 reproductive effects are considered endpoints of concern but are not considered the most
9 sensitive endpoint. As discussed further in Section 3.3, the NOAELs used for the dose response
10 assessments are substantially below the NOAEL of 10 mg/kg bw/day for reproductive effects.

11 **3.1.10. Carcinogenicity and Mutagenicity**

12 As summarized in Appendix 1 (Table A1-2), standard chronic carcinogenicity studies were
13 conducted in mice (Bond et al. 1995a, MRID 43701505) and rats (Bond et al. 1995b, MRIDs
14 43701507 and 43710503). These studies are also published in the open literature as Stebbins et
15 al. 2002 (mice) and Yano et al. 2002 (rats). The pathology and systemic toxicity noted in these
16 studies are discussed in Section 3.1.5. Neither study found any evidence of carcinogenic
17 activity. In addition, as summarized in several EPA risk assessments, spinosad was tested in a
18 variety of standard *in vitro* and *in vivo* assays for mutagenicity, and mutagenic activity is not
19 observed (U.S. EPA/OPP/HED 1997b, p. 12; U.S. EPA/OPP/HED 2009, p. 5; U.S.
20 EPA/OPP/HED 2009, p. 3). Based on these data, the EPA concludes that spinosad is “*Not likely*
21 *to be Carcinogenic to Humans*” (U.S. EPA/OPP/HED 2009a, Table A.2.1, P. 35).

22
23 Three studies on the mutagenic potential of spinosad are available in the literature from outside
24 of the United States (Aciole et al. 2014 [Brazil]; Akmoutsou et al. 2011 [Greece]; El-Hoda et al.
25 2012 [Egypt]).

26
27 Increases in number of total structural aberrations in bone marrow chromosomes were observed
28 in a subchronic study in rats (El-Hoda et al. 2012, Table 2 of study). As summarized in
29 Appendix 1 (Table A1-2), this study used a Dow AgroSciences formulation of spinosad (Tracer[®],
30 24% a.i., SC). The dietary concentrations of spinosad used in this study were 8 and 16 ppm.
31 These concentrations are substantially below the concentrations used in the chronic toxicity
32 study in rats (Bond et al. 1995b, MRIDs 43701507 and 43710503)—i.e., 50, 200, 500, or 1000
33 ppm. Given the lack of carcinogenic activity in the chronic study in rats as well as the
34 supporting study in mice, the report from El-Hoda et al. (2012) does not substantially increase
35 concern for the potential carcinogenicity of spinosad.

36
37 Exposure to spinosad (source and/or formulation not specified) resulted in an increase of
38 mutations in the somatic mutation and recombination assay in *Drosophila melanogaster* at the
39 highest concentration tested, 1.6 mg/L (Table 1 in Aciole et al. 2013). Using the same assay as
40 Aciole et al. (2013) but at lower concentrations (0.1 to 0.5 mg a.i./L of a 480 g/L formulation of
41 spinosad), Akmoutsou et al. (2011) observed no mutagenic activity in *Drosophila*. While the
42 assays in *Drosophila* are noted for the sake of completeness, this type of *in vivo* assay in an
43 insect does not raise concern for the carcinogenicity of spinosad, given the available chronic
44 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/WHO 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.

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

4
5 Given the standard assays in guinea pigs and the human experience with spinosad, there is no
6 basis for identifying skin sensitization as an endpoint of concern.

7 **3.1.11.3. Ocular Effects**

8 As with skin irritation and skin sensitization, standard assays for eye irritation are available on
9 both technical grade spinosad (MRID 43414518) and a 44% a.i. formulation of spinosad (MRID
10 43414512). These studies are summarized in Appendix 1 (Table A1-5). Slight conjunctival
11 irritation was observed in both studies. Based on the minimal responses in these studies, U.S.
12 EPA/OPP/HED categorizes both technical grade spinosad and the formulation of spinosad as
13 Category IV (i.e., the least severe category) for eye irritation (U.S. EPA/OPP/HED 1997b,
14 2009a, 2010b, 2011a).

15
16 Most of the descriptions of eye irritation studies on the MSDS for the representative formulations
17 (Table 4) are consistent with the studies summarized in Appendix 1, Table A1-5. The one
18 possible exception is Entrust [WP]. The SDS for this formulation indicates that the
19 formulation...*Causes serious eye irritation.*

20
21 The European Commission (2006) states that spinosad is not irritating to the eyes. Consistent
22 with the EPA assessment, other regulatory reviews of spinosad note that spinosad is a slight eye
23 irritant (FAO/WHO 2001; WHO 2008, 2011).

24
25 As discussed in Section 3.1.11.1, Stough et al. (2009) published a large study on the use of a
26 0.9% formulation of spinosad to treat head lice in humans. Of the 552 participants in this study
27 treated with spinosad, 12 (2.2%) reported *ocular hyperemia*—i.e., redness or inflammation of the
28 eyes (Stough et al. 2009, Table 2). This effect is noted but is not otherwise discussed in the
29 study. As also discussed in Section 3.1.11.1, this study did not involve a control group and it is
30 not clear if the response noted by Stough et al. (2009) was statistically significant. Stough et al.
31 (2009, Table 2, p. e392) provide statistics based on Fischer's Exact Test; however, these
32 statistics appear to apply to comparisons of spinosad with permethrin, another pesticide included
33 in the study. For ocular hyperemia, the reported *p*-value (0.329) would not be viewed as
34 statistically significant.

35
36 Based on the available information, minimal eye irritation might be associated with exposures to
37 spinosad; however, there is no basis for asserting that serious eye damage would be likely.

38 **3.1.12. Systemic Toxic Effects from Dermal Exposure**

39 The acute and repeated dose dermal toxicity studies on spinosad and spinosad formulations are
40 summarized in Appendix 1, Table A1-6. The acute EPA studies (MRID 43414516 and MRID
41 43557503) as well as an acute study summarized by FAO/WHO (2001) are consistent indicating
42 no observed adverse effects at doses of up to 2000 mg/kg bw in the EPA studies and 5000 mg/kg
43 bw in the study summarized by EPA. The EPA studies were used to classify spinosad as
44 Category IV (i.e., the least severe category) for acute dermal toxicity (U.S. EPA/OPP/HED
45 2011a, p. 5). All of the representative formulations explicitly covered in the current risk

1 assessment indicate dermal LD₅₀ values in rabbits of >5000 mg/kg bw. These statements are
2 consistent with the data presented in FAO/WHO 2011 (citing Stebbins and Brooks 1999a) but
3 are somewhat higher than the values of >2000 mg/kg bw/day cited in EPA documents
4 (Appendix 1, Table A1-6).

5
6 The repeated dose dermal studies contain inconsistencies. Study summaries provided in U.S.
7 EPA/OPP/HED (1992b, 2009a) and FAO/WHO (2001) indicate no adverse effects in rabbits at
8 doses of up to 1000 mg/kg bw/day with exposure periods of 6 hours/day for 21 days. An EPA
9 compendia of DERs (U.S. EPA-OPP-HED 1997a), however, contains a DER for the study by
10 Vedula and Yano (1994) in which a 43.4% formulation was assayed in rabbits at doses of 0, 100,
11 500, or 1000 mg/kg bw/day in a Phase 1 study and 0, 200, 300, or 500 mg/kg bw/day in a Phase
12 2 study. In both studies, the exposures consisted of 6 hours/day for 21-days with occlusion at the
13 application sites to minimize potential ingestion of the test compound. While no frank signs of
14 toxicity were noted, hyperplasia of the gastric mucosa was observed at doses of 300, 400, 500, or
15 1000 mg/kg bw/day. Although the responses at 300 mg/kg bw/day were not statistically
16 significant, the responses at 400, 500, and 1000 mg/kg bw/day were statistically significant using
17 the Fischer's Exact Test. It should be noted one study from (FAO/WHO 2001) is cited as
18 Vedula and Yano (1994) with a study number of DR-0323-1194-018. This study is not identical
19 to the DER of Vedula and Yano (1994) which has study numbers of DR-0341-0784-002 and
20 DR-0341-0784-002R. It should also be noted that the EPA human health risk assessments (U.S.
21 EPA/OPP/HED 1997b, 2009a, 2010b, 2011a) do not include a discussion of Vedula and Yano
22 (1994).

23
24 The above discrepancies are noted only for the sake of completeness. As discussed in Section
25 3.1.3.2.1, U.S. EPA/OPP/HED does not explicitly consider dermal exposures in their risk
26 assessments for spinosad, in part, due to the lack of toxicity observed in the repeated dose dermal
27 studies. While the above discrepancies do not have a substantial impact on this decision, the
28 current risk assessment does explicitly and quantitatively consider dermal exposures for both
29 workers and members of the general public (Section 3.2).

30 **3.1.13. Inhalation Exposure**

31 The U.S. EPA typically requires short-term (single 4-hour exposure) inhalation toxicity studies
32 in rats (U.S. EPA/OPPTS 1998b) to support pesticide registration. As summarized in
33 Appendix 1 (Table A1-7), these standard studies are available for technical grade spinosad
34 (MRID 43414517) as well as an unspecified 44% a.i. formulation of spinosad (MRID
35 43414511). The EPA may sometimes require subchronic inhalation studies (U.S. EPA/OPPTS
36 1998c), but these studies have not been required for spinosad.

37
38 The EPA documents report indefinite acute LC₅₀ values of >5.18 mg/L for technical grade
39 spinosad (MRID 43414517) and >5 mg/L for an unspecified 44% a.i. formulation (MRID
40 43414511). As noted in Table 2, the spinosyns have low vapor pressures – i.e., 2 to 3 × 10⁻⁵ mPa
41 at 25 °C. While details of the inhalation toxicity studies cited by EPA are not available,
42 concentrations in the range of 5 mg/L are not attainable in vapor form but must involve aerosols.
43

44 As summarized in Table 4, MSDS for most of the representative formulations explicitly covered
45 in the current risk assessment report LC₅₀ values over a similar range—i.e., from >4.19 to >5.51
46 mg/L. The SDS for Conserve SC reports a higher indefinite LC₅₀ of >17.02 mg/L. This value,

1 however, is specified on the SDS as applying to the formulation (11.6% a.i.). Thus, the LC₅₀
2 corresponds to about >2 mg a.i./L [17.02 mg/L x 0.116 = 1.97432]. Since all of these LC₅₀
3 values are indefinite, the differences in the values simply reflect differences in the experimental
4 concentration(s) used in the assays and do not necessarily reflect any differences in potency
5 among the different formulations.

6
7 Based on the available inhalation bioassays, the EPA classifies spinosad at Category IV for acute
8 inhalation exposure—i.e., the least hazardous ranking (U.S. EPA/OPP/HED 2011a, p. 5). As
9 discussed further in Section 3.2.2 (worker exposure), the U.S. EPA explicitly considers
10 inhalation as a route of concern for occupational exposures in their more recent risk assessments
11 (e.g., U.S. EPA/OPP/HED 2009a). This approach differs from the determination in earlier EPA
12 risk assessments that...*Exposure via inhalation is not a concern* (U.S. EPA/OPP/HED 1997b, p.
13 14). As with most Forest Service risk assessments, the occupational exposure assessments for
14 workers in the current risk assessment are based on biomonitoring studies which implicitly
15 consider all routes of exposure (i.e., dermal, inhalation, and incidental oral routes).

16 **3.1.14. Adjuvants and Other Ingredients**

17 **3.1.14.1. Other Ingredients**

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

26
27 The identities of inerts in pesticide formulations are generally considered trade secrets and need
28 not be disclosed to the general public. Nonetheless, all inert ingredients as well as the amounts
29 of the inerts in the formulations are disclosed to and reviewed by the U.S. EPA as part of the
30 registration process. Some inerts are considered potentially hazardous and are identified as such
31 on various lists developed by the federal government and state governments. Material Safety
32 Data Sheets or Safety Data Sheets sometimes specify inerts used in pesticide formulations. U.S.
33 EPA/OPP (2015b, p. 5-13) encourages but does not generally require expanded inert statements
34 on product labels which specifically identify the inert ingredients in the product. One notable
35 exception, however, involves petroleum distillates including xylene or xylene range solvents that
36 are part of the formulation and at a concentration of $\geq 10\%$. In this case, the product label must
37 contain the following statement: *Contains petroleum distillates, xylene or xylene range aromatic*
38 *solvents* (U.S. EPA/OPP 2010d, p. 5-11). None of the product labels for the representative
39 formulations listed in Table 3 indicates that these formulations contain petroleum distillates at or
40 above 10% of the formulation.

41
42 The U.S. EPA classifies inerts into one of four lists based on the available toxicity information:
43 toxic (List 1), potentially toxic (List 2), unclassifiable (List 3), and non-toxic (List 4). List 4 is
44 subdivided into two categories, 4A and 4B. List 4A constitutes inerts for which there is adequate
45 information to indicate a minimal concern. List 4B constitutes inerts for which the use patterns

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

4
5 As summarized in Table 3, the inerts specified on the MSDS/SDS for the representative
6 formulations explicitly considered in the current risk assessment include propylene glycol,
7 kaolin, and silica gel. Several of the formulations in Table 3 indicate that the formulations
8 contain propylene glycol. Propylene glycol is a List 4B inerts and is exempt from tolerances as a
9 food-use inert ingredient under the Code of Federal Regulations (40 CFR part 180). Kaolin
10 (1332-58-7) is a form of clay. Silica gel and clay are categorized at a List 4A inerts—i.e., inerts
11 of minimal concern.

12
13 For all of the formulations listed in Table 3, the percentage of spinosad combined with the
14 percentages of inerts do not total to 100%. In other words, there are unspecified inerts in all of
15 the formulations. Nonetheless, as noted above, all inerts are disclosed to and approved by the
16 U.S. EPA. One inert often not listed on MSDS/SDS is water. While speculative, it seems
17 reasonable to suggest that at least some of the proportion of undisclosed inerts in the
18 formulations listed in Table 3 may consist of water.

19 **3.1.14.2. Adjuvants**

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

27
28 The product labels for representative formulations of spinosad (Table 3) indicate that emulsified
29 or methylated crop oil as well as organosilicone surfactants may be used in applications for the
30 control of some pests, however, fuel oil and mineral oil should not be used.

31 **3.1.15. Impurities and Metabolites**

32 **3.1.15.1. Metabolites**

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

39
40 The *in vivo* metabolites of spinosad are discussed in Section 3.1.3.1, and an overview of these
41 metabolites is given in Figure 4. As illustrated in Figure 4, the major mammalian *in vivo*
42 metabolites involve N- and O-demethylation as well as conjugation with glutathione. The
43 environmental metabolism of spinosad is reviewed in detail by Cleveland et al. (2002a, see
44 Figure 1 of their paper) and Mandal et al. (2013, see Figure 2 of their paper). The environmental

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

8
9 From a practical perspective, metabolites have an impact on the risk assessment when they are of
10 comparable or greater toxicity than the parent compound. For spinosad, there is no indication
11 that the metabolites are much more toxic to mammals than the parent compounds (spinosyns A
12 and D). As discussed further in Section 4.1.3.3, this is not the case for aquatic invertebrates,
13 which does not have an impact on the hazard identification for human health effects. No
14 information is available on the toxicity of the environmental metabolites to humans or
15 experimental mammals. Noting the structural similarity of most metabolites of spinosad to the
16 parent compounds, U.S. EPA/OPP/HED (2011a, p. 9) adopts a total residue approach for aquatic
17 modeling. Essentially, this method assumes that the toxicities of the metabolites are comparable
18 to the toxicities of the parent compounds. This approach is also adopted in the current risk
19 assessment, as detailed further in Section 3.2.3 (exposure assessments for members of the
20 general public).

21 **3.1.15.2. Impurities**

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

29
30 Registrants disclose the nature of impurities in their technical grade material to the U.S. EPA;
31 however, the identities of the impurities are not disclosed to the public, because that information
32 may provide insight into the manufacturing process, which is considered proprietary and is
33 protected under FIFRA (Section 10). Proprietary information on the identities of these
34 impurities was not available for the preparation of the current Forest Service risk assessment.

35
36 To some extent, concern for impurities in technical grade spinosad is reduced because most of
37 the existing toxicity studies were conducted with the technical grade product. Thus, any toxic
38 impurities present in the technical grade product are likely to be encompassed by the available
39 toxicity studies on the technical grade product.

40 **3.1.16. Toxicologic Interactions**

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

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

4
5 In studies on the joint action of spinosad and milbemycin oxime, both of which are used to treat
6 fleas in dogs, Holstrom et al. (2012) note that co-exposure to spinosad increases systemic levels
7 of milbemycin oxime. While not providing detailed experimental data, these investigators
8 suggest that this interaction may be due to decreased metabolism of milbemycin oxime by
9 cytochrome P450. Cytochrome P-450 is a general term for a class of mixed function oxidases
10 involved in the metabolism of a broad range of naturally occurring chemicals (e.g., steroids) as
11 well as xenobiotics (i.e., man-made chemicals typically not found in nature). In general, any
12 compound that inhibits a mixed function oxidase may inhibit or alter the metabolism of other
13 compounds that also serve as substrates for the mixed function oxidase. Furthermore, substrates
14 for mixed function oxidases can often induce the production of mixed function oxidases, thereby
15 enhancing their own metabolism as well as that of other compounds (e.g., Coon 2005; Lewis et
16 al. 1998). Depending on the compounds involved in these interactions, the toxicity of the
17 compounds could be enhanced (if P-450 detoxifies the compounds) or reduced (if P-450
18 metabolizes the compounds to more toxic metabolites). As discussed further in Section
19 4.1.2.4.1, the available information on insects clearly indicates that metabolism by cytochrome
20 P-450 is a detoxification mechanism in terrestrial insects. Comparable data in mammals has not
21 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.

1
2 In Table 14 of SERA (2014b), three reference chemicals with corresponding worker exposure
3 rates are given for directed foliar applications with differing first-order dermal absorption rate
4 coefficients (k_a values)—i.e., glyphosate ($k_a = 0.00041 \text{ hour}^{-1}$), 2,4-D ($k_a = 0.00066 \text{ hour}^{-1}$), and
5 triclopyr BEE ($k_a = 0.0031 \text{ hour}^{-1}$). As discussed in Section 3.1.3.2.2 of the current risk
6 assessment, the central estimate of the first-order dermal absorption rate coefficient for spinosad
7 is $0.00002 \text{ hour}^{-1}$. This rate coefficient for spinosad is about a factor of about 20 less than the
8 corresponding coefficient for glyphosate, the reference pesticide with the lowest k_a [0.00041
9 $\text{hour}^{-1} \div 0.00002 \text{ hour}^{-1} = 20.5$]. While a factor of 20 involves substantial extrapolation,
10 glyphosate is used as the reference chemical for directed foliar applications in order to minimize
11 extrapolation. For directed foliar applications, the application of the methodology from SERA
12 (2014b) is detailed in Table 5. The rates given in Table 5 are rounded to two significant digits
13 and are used in Worksheet C01a the attachments to the risk assessment to estimate exposures for
14 workers involved in directed foliar applications.

15
16 As also summarized in Table 14 of SERA (2014b), only one reference chemical, 2,4-D, is
17 available for ground broadcast and aerial applications, and the first-order dermal absorption rate
18 coefficient for 2,4-D is taken as $0.00066 \text{ hour}^{-1}$. This first-order dermal absorption rate
19 coefficient is below the corresponding value for spinosad by a factor of over 30 [$0.00066 \text{ hour}^{-1} \div$
20 $0.00002 \text{ hour}^{-1} \approx 33.0033$]. While the application of dermal adjustment factors is optional in the
21 SERA (2014b) methodology, the dermal adjustment factor is used in this risk assessment of
22 spinosad. The most recent EPA occupational exposure assessments do not specifically consider
23 dermal absorption (U.S. EPA/OPP/HED 2009a, Table 7.1.1, p. 37). As noted in Section
24 3.1.3.2.1 (First-Order Dermal Absorption), not addressing dermal absorption reflects the EPA's
25 assessment of the poor dermal absorption and low dermal toxicity of spinosad. Given the
26 approach taken by EPA and for the same reasons—i.e., apparent poor dermal absorption and low
27 dermal toxicity—the application of the dermal absorption adjustment factor for spinosad seems
28 reasonable. These adjustments are detailed in Table 8 (ground broadcast applications) and Table
29 9 (aerial applications).

30
31 In addition to the application rate and absorbed dose rate, the other factor affecting worker
32 exposure is the number of acres per day that a worker will treat, in that acres treated per day are
33 used in estimating the amount of pesticide that a worker will handle. Estimates of the number of
34 acres per day that a worker might treat are taken from SERA (2014b, Table 2 and Section 1.1).
35 These estimates are as important as worker exposure rates, and estimates of the number of acres
36 treated per day should be adjusted as appropriate for any site-specific application.

37 **3.2.2.2. Accidental Exposures**

38 Generally, dermal exposure is the predominant route of exposure for pesticide applicators
39 (Ecobichon 1998; van Hemmen 1992); hence, accidental dermal exposures are considered
40 quantitatively in all Forest Service risk assessments. The two types of dermal exposures
41 modeled in the risk assessments include direct contact with a pesticide solution and accidental
42 spills of the pesticide onto the surface of the skin. In addition, two exposure scenarios are
43 developed for each of the two types of dermal exposure, and the estimated absorbed dose for
44 each scenario is expressed in units of mg chemical/kg body weight. Both sets of exposure
45 scenarios are summarized in Worksheet E01 of the EXCEL workbooks that accompany this risk

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

3
4 Exposure scenarios involving direct contact with solutions of spinosad are characterized either
5 by immersion of the hands in a field solution for 1 minute or wearing pesticide contaminated
6 gloves for 1 hour. The assumption that the hands or any other part of a worker's body will be
7 immersed in a chemical solution for a prolonged period of time may seem unreasonable;
8 however, it is possible that the gloves or other articles of clothing worn by a worker may become
9 contaminated with pesticide resulting in potentially long periods of exposure. For these exposure
10 scenarios, the key assumption is that wearing gloves grossly contaminated with a chemical
11 solution is equivalent to immersing the hands in the solution. In both cases, the chemical
12 concentration in contact with the skin and the resulting dermal absorption rate are essentially
13 constant. For both scenarios (hand immersion and contaminated gloves), the assumption of zero-
14 order absorption kinetics is appropriate. For these types of exposures, the rate of absorption is
15 estimated based on a zero-order dermal absorption rate (K_p). Details regarding the derivation of
16 the K_p value for spinosad are provided in Section 3.1.3.2.2. The amount of the pesticide
17 absorbed per unit time depends entirely on the concentration of the chemical in solution. This
18 concentration is highly variable depending on the application method and also on the dilution
19 volumes, as discussed in Section 2.4. These exposure scenarios are detailed in Worksheets C02a
20 (1-minute exposure) and C02b (60-minute exposure).

21
22 The details of the accidental spill scenarios for workers consist of spilling a chemical solution on
23 to the lower legs as well as spilling a chemical solution on to the hands, at least some of which
24 adheres to the skin. The absorbed dose is then calculated as the product of the amount of
25 chemical on the skin surface (i.e., the amount of liquid per unit surface area multiplied by the
26 surface area of the skin over which the spill occurs and the chemical concentration in the liquid),
27 the first-order absorption rate coefficient, and the duration of exposure. The first-order dermal
28 absorption rate coefficient (k_a) is derived in Section 3.1.3.2.1. These exposure scenarios are
29 detailed in Worksheets C03a (spill on to the hand) and C03b (spill onto the lower legs).

30 **3.2.3. General Public**

31 ***3.2.3.1. General Considerations***

32 **3.2.3.1.1. Likelihood and Magnitude of Exposure**

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

40
41 Because of the conservative exposure assumptions used in the current risk assessment, neither
42 the probability of exposure nor the number of individuals who might be exposed has a
43 substantial impact on the characterization of risk presented in Section 3.4. As detailed in SERA
44 (2014a, Section 1.2.2.2), the exposure assessments developed in this risk assessment are based

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

5
6 This Extreme Value approach is essentially an elaboration on the concept of the *Most Exposed*
7 *Individual* (MEI), sometime referred to as the *Maximum Exposed Individual* (MEI). As this
8 name also implies, exposure assessments that use the MEI approach are made in an attempt to
9 characterize the extreme but still plausible upper bound on exposure. This approach is common
10 in exposure assessments made by U. S. EPA, other government agencies, and other
11 organizations. In the current risk assessment and other Forest Service risk assessments, the
12 upper bounds on exposure estimates are all based on the MEI.

13
14 In addition to this upper bound MEI value, the Extreme Value approach used in this risk
15 assessment provides a central estimate of exposure as well as a lower bound on exposure. While
16 not germane to the assessment of upper bound risk, it is significant that the use of the central
17 estimate and especially the lower bound estimate is not intended to lessen concern. To the
18 contrary, the central and lower estimates of exposure are used to assess the feasibility of
19 mitigation—e.g., protective measures to limit exposure. If lower bound exposure estimates
20 exceed a level of concern, this is strong indication that the pesticide cannot be used in a manner
21 that will lead to acceptable risk.

22 **3.2.3.1.2. Summary of Assessments**

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

27
28 For spinosad, a standard set of exposure assessments used in all Forest Service risk assessments
29 for broadcast applications is considered. As summarized in Worksheet E03 of Attachments 1
30 and 2, the kinds of exposure scenarios developed for the general public include acute accidental,
31 acute non-accidental, and longer-term or chronic exposures. The accidental exposure scenarios
32 assume that an individual is exposed to the compound of concern either during or shortly after its
33 application. Non-accidental exposures involve dermal contact with contaminated vegetation as
34 well as the consumption of contaminated fruit, vegetation, water, or fish. The longer-term or
35 chronic exposure scenarios parallel the acute exposure scenarios for the consumption of
36 contaminated fruit, water, or fish. All of the non-accidental exposure scenarios are based on
37 levels of exposure to be expected following an application of spinosad at 0.225 lb a.i./acre
38 (Attachment 1) or two applications at 0.225 lb a.i./acre with a 6-day application interval
39 (Attachment 2). The upper bounds of the exposure estimates for the non-accidental scenarios
40 involve conservative assumptions intended to reflect exposure for the MEI (*Most Exposed*
41 *Individual*). The impact on the risk characterization of lower application rates is discussed in
42 Section 3.4.

43
44 The nature of the accidental exposure scenarios is intentionally extreme. The non-accidental,
45 acute exposure scenarios are intended to be conservative but plausible, meaning that it is not
46 unreasonable to assume that the magnitude of exposures in the non-accidental exposure scenarios

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

8 **3.2.3.2. Direct Spray**

9 Direct spray scenarios for members of the general public are modeled in a manner similar to
10 accidental spills for workers (Section 3.2.2.2). In other words, it is assumed that the individual is
11 sprayed with a field solution of the compound and that some amount of the compound remains
12 on the skin and is absorbed by first-order kinetics. Two direct spray scenarios are given, one for
13 a young child (D01a) and the other for a young woman (D01b).
14

15 For the young child, it is assumed that a naked child is sprayed directly during a broadcast
16 application and that the child is completely covered with pesticide (i.e., 100% of the surface area
17 of the body is exposed). This exposure scenario is intentionally extreme. As discussed in
18 Section 3.2.3.1.1, the upper limits of this exposure scenario are intended to represent the *Extreme*
19 *Value* of exposure for the *Most Exposed Individual* (MEI).
20

21 The exposure scenario involving the young woman (Worksheet D01b) is somewhat less extreme,
22 but more plausible, and assumes that the woman is accidentally sprayed over the feet and lower
23 legs. By reason of allometric relationships between body size and dose-scaling, a young woman
24 would typically be subject to a somewhat higher dose than would the standard 70 kg man.
25 Consequently, in an effort to ensure a conservative estimate of exposure, a young woman, rather
26 than an adult male, is used in many of the exposure assessments.
27

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

33 **3.2.3.3. Dermal Exposure from Contaminated Vegetation**

34 In this exposure scenario, it is assumed that spinosad is sprayed on to vegetation and that a young
35 woman comes in contact with sprayed vegetation or other contaminated surfaces at some period
36 after the spray operation (D02). For these exposure scenarios, some estimates of dislodgeable
37 residue (a measure of the amount of the chemical that could be freed from the vegetation) and
38 the rate of transfer of the chemical from the contaminated vegetation to the surface of the skin
39 must be available.
40

1 No data are available on dermal transfer rates for spinosad. This is not a severe limitation in this
2 risk assessment. As detailed in Durkin et al. (1995), dermal transfer rates are reasonably
3 consistent for numerous pesticides, and the methods and rates derived in Durkin et al. (1995) are
4 used as defined in Worksheet D02. Similarly, no data are available on dislodgeable residues for
5 spinosad. Again citing the low dermal toxicity of spinosad, U.S. EPA/OPP (2015c, p. 80669)
6 indicates that ...*dislodgeable-foliar residue (DFR) studies are unnecessary at this time as there*
7 *is no hazard via the dermal route of exposure.* In the absence of data, a default dislodgeable
8 residue rate of 0.1 of the nominal application rate is used for this exposure scenario.
9

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

14 **3.2.3.4. Contaminated Water**

15 **3.2.3.4.1. Accidental Spill**

16 The accidental spill scenario assumes that a young child consumes contaminated water shortly
17 after an accidental spill of a field solution into a small pond. The calculation of the concentration
18 of spinosad in water following the spill is given in Worksheet B04b, and the estimate of the dose
19 to a small child is given in Worksheet D05 of the attachments to this risk assessment. Because
20 this scenario is based on the assumption that exposure occurs shortly after the spill, no
21 dissipation or degradation is considered.
22

23 Since this exposure scenario is based on assumptions that are somewhat subjective and highly
24 variable, the scenario may overestimate exposure. The actual chemical concentrations in the
25 water will vary according to the amount of compound spilled, the size of the water body into
26 which it is spilled, the time at which water consumption occurs relative to the time of the spill,
27 and the amount of contaminated water that is consumed. All Forest Service risk assessments
28 assume that the accidental spill occurs in a small pond with a surface area of about one-quarter of
29 an acre (1000 m²) and a depth of 1 meter. Thus, the volume of the pond is 1000 m³ or 1,000,000
30 liters.
31

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

40 **3.2.3.4.2. Accidental Direct Spray/drift for a Pond or Stream**

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

1 detailed in Worksheet B04c (small pond) and Worksheet B04d (small stream). As would be
2 expected, the concentrations for direct spray are far below the concentrations associated with the
3 accidental spill—i.e., about 0.025 mg/L for a small pond and 0.02 mg/L for a small stream.
4 Also, as expected, the concentrations associated with drift are much lower. Using a distance of
5 25 feet down wind as examples, the concentrations in a small pond and a small stream are about
6 0.0002 to 0.004 mg/L, depending on the application method.

7 **3.2.3.4.3. GLEAMS Modeling**

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

13
14 Gleams-Driver offers the option of conducting exposure assessments using site-specific weather
15 files from Cligen, a climate generator program developed and maintained by the USDA
16 Agricultural Research Service (USDA/NSERL 2004). Gleams-Driver was used in the current
17 risk assessment to model spinosad concentrations in a small stream and a small pond.

18
19 As summarized in Table 10, nine locations are used in the Gleams-Driver modeling. These
20 locations are standard sites used in Forest Service risk assessments for Gleams-Driver
21 simulations and are intended to represent combinations of precipitation (dry, average, and wet)
22 and temperature (hot, temperate, and cool) (SERA 2007a). The characteristics of the fields and
23 bodies of water used in the simulations are summarized in Table 11. For each location,
24 simulations were conducted using clay (high runoff, low leaching potential), loam (moderate
25 runoff and leaching potential), and sand (low runoff, high leaching potential) soil textures. For
26 each combination of location and soil, Gleams-Driver was used to simulate pesticide losses to
27 surface water from 100 modeled applications at a unit application rate of 1 lb a.i./acre, and each
28 of the simulations was followed for a period of about 1½ years post application. Note that an
29 application rate of 1 lb a.i./acre is used as a convention in all Forest Service risk assessments in
30 order to avoid rounding limitations in GLEAMS outputs and are referred to as water
31 contamination rates (WCR), concentrations in water associated with an application rate of 1
32 lb/acre. In the workbooks that accompany this risk assessment, the WCRs are converted to
33 expected concentrations by multiplying the WCRs by the anticipated application rate of 0.225 lb
34 a.i./acre as discussed in Section 2 (Program Description). As also discussed in Section 2,
35 separate simulations are run for a single application (Appendix 8) and two applications with an
36 application interval of 6 days (Appendix 9).

37
38 Table 12 summarizes the chemical-specific values used in Gleams-Driver simulations. For the
39 most part, the chemical properties used in the Gleams-Driver simulations are based on the
40 parameters used by the Environmental Fate and Effects Division (EFED) of the U.S. EPA's
41 Office of Pesticides Programs modeling of spinosad (U.S. EPA/OPP/EFED 2009a). One
42 substantial difference between the EPA and GLEAMS-Driver modeling involves estimates of
43 variability. The EPA modeling is typically based on either central estimates or upper bound (90th
44 percentile) input parameters. Following the Extreme Value approach discussed in Section
45 3.2.3.1.1, the input parameters for the GLEAMS-Driver modeling are based on estimates of
46 variability either as ranges or confidence intervals when estimates of variability are available.

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

18
19 Table 13 summarizes the modeled concentrations of spinosad in surface water by GLEAMS-
20 Driver. Details of the GLEAMS-Driver simulations are detailed in Appendix 7 for a single
21 application and Appendix 8 two applications with a 6-day application interval. Note that the
22 concentrations modeled for two applications with an application interval of 6 days are
23 approximately twice those of a single application. This relationship follows from the essential
24 stability of spinosad and spinosad metabolites as discussed above. The specific concentrations of
25 spinosad in surface water used in the exposure assessments for the current risk assessment are
26 discussed in Section 3.2.3.4.6, following a comparison of the GLEAMS-Driver simulations with
27 surface water models used by EPA (Section 3.2.3.4.4).

28 **3.2.3.4.4. Other Modeling Efforts**

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

37
38 FIRST (*FQPA Index Reservoir Screening Tool*) is a Tier I (i.e., screening level) model
39 developed by the EPA for estimating concentrations of pesticides in surface water (U.S.
40 EPA/OPP 2008). As with the GLEAMS-Driver modeling and for the same reasons (Section
41 3.2.3.4.3), the concentrations estimated by FIRST for two applications are about twice those
42 estimated for a single application. Consequently, only the single application comparisons are
43 discussed.

44
45 Based on the central estimates of exposure, the estimated concentrations from FIRST are similar
46 to those for GLEAMS-Driver based on clay soils. The peak central estimate from FIRST is 23

1 $\mu\text{g/L}$ versus an estimated peak concentration from GLEAMS-Driver of $18.7 \mu\text{g/L}$. The longer-
2 term estimate from FIRST is $6.5 \mu\text{g/L}$ versus an estimated longer-term concentration from
3 GLEAMS-Driver of $6.05 \mu\text{g/L}$. The range of concentrations from the GLEAMS-Driver
4 modeling for both clay and loam soils encompass the PRZM/EXAMS simulations for bulb
5 vegetables from U.S. EPA/OPP/EFED (2005). The ranges from the GLEAMS-Driver modeling
6 (e.g., $1.6\text{-}172 \mu\text{g/L}$ per lb/acre for peak concentrations in clay soils), however, are much greater
7 than those from the FIRST modeling (i.e., $17\text{-}43.4 \mu\text{g/L}$ per lb/acre). Broader ranges from the
8 GLEAMS-Driver modeling relative to both FIRST and PRZM/EXAMS modeling are commonly
9 noted in Forest Service risk assessments and appear to reflect the broader range of input values
10 used in the GLEAMS-Driver modeling, the number and diversity of locations and soil types used
11 in the GLEAMS-Driver modeling, and the large number of simulations conducted in the
12 GLEAMS-Driver modeling relative to the PRZM/EXAMS modeling.

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

21 **3.2.3.4.5. Monitoring Data**

22 No monitoring data for spinosad are included in compendia published by the U.S. Geological
23 Survey's National Water-Quality Assessment Program (USGS/NAWQA) covering periods from
24 1992-2001 (Gilliom et al. 2007) or the more recent update covering periods from 1992-2008
25 (Ryberg et al. 2011). In the conduct of the current Forest Service risk assessment, the California
26 database (<http://www.cdpr.ca.gov/docs/emon/surfwtr/surfcont.htm>) was searched (April 5, 2016)
27 and no monitoring data were identified. Monitoring studies are not discussed in the EPA or
28 APHIS risk assessments on spinosad (Table 1, Section 1.1).

29
30 The one available monitoring study from the California Department of Pesticide Regulation (Fan
31 et al. 2008) is associated with the application of spinosad for the eradication of the Mexican fruit
32 fly in San Diego County during 2003. The application involved a 23 square mile area treated at
33 a rate of $3.26 \mu\text{g}/\text{ft}^2$ or about $0.0003 \text{ lb a.i./acre}$ [$3.26 \mu\text{g}/\text{ft}^2 \times 43560 \text{ ft}^2/\text{acre} = 142,005.6 \mu\text{g}/\text{acre}$
34 $\approx 0.000142 \text{ kg}/\text{acre}$; $0.000142 \text{ kg}/\text{acre} \times 2.2046 \text{ lb}/\text{kg} \approx 0.0003 \text{ lb}/\text{acre}$]. Spinosad was not
35 detected in surface water or rain runoff, which is to be expected, given the low application rate.
36 As summarized in Table 13, the direct spray of a small pond at an application rate of 1 lb
37 a.i./acre could result in a concentration of $112 \mu\text{g/L}$. At an application rate of $0.0003 \text{ lb}/\text{acre}$, the
38 expected concentration would be about $0.03 \mu\text{g/L}$ [$112 \mu\text{g/L} \times 0.0003 \text{ lb}/\text{acre} \approx 0.0336 \mu\text{g/L}$].
39 This concentration is somewhat below the detection limit of $0.05 \mu\text{g/L}$ for spinosad in water
40 noted by Fan et al. (2008, p. 18).

41
42 In terms of evaluating the surface water modeling efforts discussed in the previous sections, the
43 most useful monitoring studies are those that associate monitored concentrations of a pesticide in
44 water with defined applications of the pesticide—e.g., applications at a defined application rate
45 to a well characterized field. When available, such studies can provide a strong indication of the
46 plausibility of modeled concentrations of a pesticide in surface water. In the absence of

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

3 **3.2.3.4.6. Concentrations in Water Used for Risk Assessment**

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

18
19 As discussed previously and summarized in Table 13, the Gleams-Driver simulations of the
20 small pond provide the highest estimates of spinosad concentrations in surface water and the
21 central estimates from GLEAMS-Driver are reasonably consistent with the central estimates
22 from the Tier I modeling using FIRST. As detailed in Section 3.2.3.4.3, the GLEAMS-Driver
23 simulations encompass a much broader range of soils and locations with a concomitant increase
24 in the range of modelled values. Consequently, the Gleams-Driver simulations serve as the
25 primary basis for the water concentrations of spinosad used in the current risk assessment.

26
27 As noted in 3.2.3.4.5, monitoring data on concentrations of spinosad in surface water are not
28 available to assess the plausibility of the modeling. While the Gleams-Driver estimates are
29 reasonably consistent with U.S. EPA/OPP modeling (Section 3.2.3.4.4), the lack of appropriate
30 monitoring data adds uncertainty to this risk assessment.

31
32 As with all uses of GLEAMS-Driver in Forest Service risk assessments, the estimated
33 concentrations of spinosad in water cover a substantial range. For example, the estimated peak
34 concentrations following a single application range from 0.00008 to 0.17 mg/L per lb a.i.
35 applied. This range spans a factor of over 2000 [$0.17 \div 0.00008 = 2125$]. This variability is
36 typical of composite summaries of GLEAMS-Driver simulations in Forest Service risk
37 assessments and reflects the wide range of conditions used in the GLEAMS-Driver modeling
38 (Section 3.2.3.4.3). In region-specific or site-specific assessments, considerations should be
39 given to the more detailed summaries of the modeling simulations in Appendix 8 (one
40 application) or Appendix 9 (two applications) or to conducting site-specific assessments to
41 reflect local conditions (see SERA 2011b, Section 3.3.4).

42 **3.2.3.5. Oral Exposure from Contaminated Fish**

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

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

5
6 Three sets of exposure scenarios are presented: one set for acute exposures following an
7 accidental spill (Worksheets D08a and D08b), one set for acute exposures based on expected
8 peak concentrations of spinosad in water (Worksheets D09c and D09d), and another set for
9 chronic exposures based on estimates of longer-term concentrations in water (Worksheets D09a
10 and D09b). The two worksheets for each set of scenarios are included to account for different
11 consumption rates of caught fish among the general population and subsistence populations.
12 Details of these exposure scenarios are provided in Section 3.2.3.5 of SERA (2014a).

13
14 The scenarios associated with consumption of contaminated fish are based on the same
15 concentrations of spinosad in water used for the accidental spill scenario (Section 3.2.3.4.1.) and
16 the surface water exposure estimates (Section 3.2.3.4.6).

17
18 Generally, bioconcentration factors for the edible portion of fish (i.e., muscle) are used in the
19 human health risk assessment under the assumption that humans will not generally consume
20 offal. As summarized in Table 2, BCFs are available for spinosyn A (MRID 43557601),
21 spinosyn D (MRID 44537734), and total residues of spinosyns A, D, and metabolites (U.S.
22 EPA/OPP/EFED 2009a, p. 8). Consistent with the total residue approach taken for surface water
23 modeling, the current risk assessment uses the BCF for total residues—i.e., bioconcentration
24 factors of 16 to 47 for edible tissue. Given the relationship between exposure time and
25 bioconcentration, the lower bound of 16 is used for acute exposures and the upper bound of 47 is
26 used for longer-term exposures. As noted in Section 4.2.2.5, the BCFs for whole fish are used in
27 the exposure assessments for mammalian and avian wildlife—i.e., a BCF of 84 for acute
28 exposures and a BCF of 115 for longer-term exposures.

29 **3.2.3.6. Dermal Exposure from Swimming in Contaminated Water**

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

33
34 To assess the potential risks associated with swimming in contaminated water, an exposure
35 assessment is developed for a young woman swimming in surface water for 1 hour (Worksheet
36 D10). Conceptually and computationally, this exposure scenario is virtually identical to the
37 contaminated gloves scenario used for workers (Section 3.2.2.2)—i.e., a portion of the body is
38 immersed in an aqueous solution of the compound at a fixed concentration for a fixed period of
39 time.

40
41 As in the corresponding worker exposure scenario, the 1-hour period of exposure is intended as a
42 unit exposure estimate. In other words, both the absorbed dose and consequently the risk will
43 increase linearly with the duration of exposure, as indicated in Worksheet D10. Thus, a 2-hour
44 exposure would lead to an HQ that is twice as high as that associated with an exposure period of
45 1 hour. In cases in which this or other similar exposures approach a level of concern, further

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

3
4 The scenarios for exposures associated with swimming in contaminated water are based on the
5 peak water concentrations of spinosad used to estimate acute exposure to drinking water
6 (Section 3.2.3.4.6).

7 **3.2.3.7. Oral Exposure from Contaminated Vegetation**

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

14
15 For pesticides that may be applied to vegetation, Forest Service risk assessments include
16 standard exposure scenarios for the acute and longer-term consumption of contaminated
17 vegetation. Two sets of exposure scenarios are provided: one for the consumption of
18 contaminated fruit and the other for the consumption of contaminated vegetation. These
19 scenarios, detailed in Worksheets D03a (fruit) and D03b (vegetation) for acute exposure and
20 Worksheets D04a (fruit) and D04b (vegetation) for chronic exposure. The key inputs for these
21 scenarios are the initial residues on the vegetation and the amount of fruit or vegetation
22 consumed for both acute and chronic scenarios. For chronic scenarios, additional key inputs are
23 the half-lives of the pesticide on the fruit or vegetation as well as the period used to estimate the
24 average concentration of the pesticide on vegetation.

25
26 In most Forest Service risk assessments, the initial concentration of the pesticide on fruit and
27 vegetation is estimated using the empirical relationships between application rate and
28 concentration on different types of vegetation (Fletcher et al. 1994). These residue rates are
29 summarized in Table 15. The rates provided by Fletcher et al. (1994) are based on a reanalysis
30 of data originally compiled by Hoerger and Kenaga (1972) and represent estimates of pesticide
31 concentration in different types of vegetation (mg chemical/kg vegetation) at a normalized
32 application rate of 1 lb a.i./acre. Although the EPA human health risk assessments do not
33 consider exposure scenarios involving direct spray, the residue rates recommended by Fletcher et
34 al. (1994) are used by U.S. EPA/OPP/EFED in their T-REX exposure model for terrestrial
35 organisms (http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm).

36
37 Table 15 also summarizes two residue studies on spinosad, one study on cauliflower (Mandel et
38 al. 2009) and the other study on cowpea pods (Vijayasree et al. 2014). As detailed in Table 15,
39 both studies yield estimated residue rates of about 15 mg a.i./kg food) per lb a.i./acre. The study
40 by Vijayasree et al. (2014) on cowpea pods is consistent with the upper bound estimate of 15 mg
41 a.i./kg food) per lb a.i./acre for fruits, pods, seeds, and large insects from Fletcher et al. (1994).
42 Cauliflower is essentially a variety of cabbage, which would typically be classified as a
43 broadleaf. The residue rates for cauliflower of about 14 to 16 a.i./kg cauliflower from Mandel et
44 al. (2009) are near the lower bound for broadleaf plants from Fletcher et al. (1994). While based
45 on only two studies, the reasonable concordance of pesticide-specific residues rates with the rates
46 from Fletcher et al. (1997) is a common pattern noted in Forest Service risk assessments. This

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

7
8 The half-lives on vegetation used in chronic exposure scenarios are based on the same rates used
9 in GLEAMS-Driver modeling (Table 12)—i.e., 6 days with a range of 1.5 to 35 days. The
10 central estimate is approximated from Sharma et al. (2008, high application rate) and lower
11 bound values from Tomkins et al. (1991). As summarized in Table 2, several foliar half-lives are
12 reported in the open literature with values ranging from about 1.5 days (Mandal et al. 2009;
13 Sharma et al. 2008; Singh and Battu 2012) to about 16 days (Tomlin 2004; Tomkins et al. 1991).
14 The lower bound half-life of 1.5 days is taken from Vijayasree et al. (2014, cowpea). While a
15 half-life of 16 days could be used based on the values for spinosad reported in the literature, the
16 upper bound value of 35 days is taken in deference to U.S. EPA/OPP/EFED (2011a), which uses
17 a default half-life of 35 days in an ecological risk assessment on spinosad.

18
19 Based on these half-lives on vegetation and fruit, the longer-term concentrations of the pesticide
20 in various commodities are detailed in Worksheets B05a (fruit), B05b (broadleaf vegetation),
21 B05c (short grass), and B05d (long grass). Only the worksheets for fruit and broadleaf
22 vegetation are used in the human health risk assessment. All four worksheets are used in the
23 ecological risk assessment (Section 4.2). In all cases, a maximum 90-day time-weighted average
24 concentration is calculated for longer-term exposures. In the context of the human health risk
25 assessment, the use of the 90-day rather than a 365-day time-weighted average is intended to
26 reflect the harvesting of a 1-year supply of fruit and/or vegetation during a single season (i.e.,
27 about 90 days) under the assumption that degradation will not occur once the commodity is
28 harvested—e.g., the commodities are placed in cold storage, which essentially stops the
29 degradation of the pesticide.

30
31 As summarized in Worksheet E03 of Attachment 1 (single application), the estimated acute
32 exposures are 0.00265 (0.00121 – 0.042) mg/kg bw for the consumption of contaminated fruit
33 and 0.0365 (0.00253-0.34) mg/kg bw/day for the consumption of contaminated vegetation. The
34 estimated longer-term exposures are 0.000254 (0.0000291-0.0196) mg/kg bw/day for
35 contaminated fruit and 0.00351 (0.000061-0.142) mg/kg bw/day for contaminated vegetation.
36 As summarized in Worksheet E03 of Attachment 2 (two applications), the estimated doses for
37 contaminated fruit and vegetation are somewhat less than a factor of 2 higher than the doses
38 associated with a single application. This is to be expected given the short interval between
39 applications (i.e., 6 days).

40
41 The U.S. EPA/OPP approach to dietary exposure is different from the approach used in Forest
42 Service risk assessments. While Forest Service risk assessments consider the consumption of
43 fruit and vegetation directly sprayed with a pesticide, the EPA exposure assessments are based
44 on dietary surveys (i.e., the amounts of different commodities consumed by individuals) and
45 tolerance limits on those commodities—i.e., the concentration of vegetation used in the exposure
46 assessment assumes that the tolerances set by EPA are not exceeded. In EPA's most recent

1 human health risk assessment document (U.S. EPA/OPP/HED 2011a, Table A.7.1, p. 60),
2 estimates of total chronic dietary exposures for humans of different age groups range from about
3 0.0018 to 0.0059 mg/kg bw/day. These estimates are similar to the central estimates of chronic
4 exposures derived in the current risk assessment for the consumption of contaminated vegetation.
5 This similarity, however, is coincidental. More significantly in terms of the interpretation of
6 potential risk, the upper bound estimates of dietary exposure given the current risk assessment
7 are substantially higher than those presented by EPA. This is a common pattern in Forest
8 Service risk assessments and reflects the different methods and scenarios used in Forest Service
9 risk assessments, relative to the methods used by EPA.
10

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

1 (2001, p. 53) review of spinosad: *In studies with repeated doses, no acute toxicological alerts*
2 *were observed that might indicate the need for establishing an acute reference dose.*
3

4 As detailed in Section 3.2, several accidental and non-accidental exposure scenarios typically
5 used in Forest Service risk assessments are developed for spinosad. All of these exposure
6 assessments involve exposure for a single day or during a single incident. In the absence of an
7 acute RfD associated with a single day or single incident exposure, the current Forest Service
8 risk assessment uses the approach developed by EPA for short-term incidental exposures (1-30
9 days). The U.S. EPA/OPP/HED (2009a, Table 3.1, p. 21) assesses such short-term incidental
10 exposures using the NOAEL of 4.9 mg/kg bw/day from a 90-day feeding study in dogs (i.e.,
11 MRID 43444102 as summarized in Appendix 1, Table A1-2). In applying this NOAEL to risk
12 characterization, the EPA uses a Margin of Exposure (MOE) of 100, which is based on a factor
13 of 10 for extrapolating from animals to humans multiplied by a factor of 10 considering sensitive
14 subgroups in the human population, which is fundamentally equivalent to a short-term RfD of
15 0.049 mg/kg bw. This approach is maintained by EPA in their most recent human health risk
16 assessment scoping document (U.S. EPA/OPP/HED 2011a, Table A.2.1). In the absence of an
17 acute RfD, the short-term equivalent RfD of 0.049 mg/kg bw is used to characterize risks
18 associated with acute exposures in the current risk assessment.
19

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

23 **3.3.3. Chronic RfD**

24 No chronic RfD for spinosad is available at the EPA's Integrated Risk Information System
25 (IRIS) (<https://www.epa.gov/iris>). U.S. EPA/OPP derives two chronic RfDs for spinosad. An
26 ADI for spinosad is also derived by EFSA (2013).
27

28 Originally, the EPA derived a chronic RfD of 0.0268 mg/kg bw/day based on the chronic study
29 of spinosad in dogs (U.S. EPA/OPP/HED 1997b, p. 13). As summarized in Appendix 1, Table
30 A1-2, this study defines a NOAEL of 2.68 mg/kg bw/day and a LOAEL of 8.36 mg/kg bw/day
31 based on changes in clinical chemistries and tissue pathology (Harada 1995, MRID 43701504).
32

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

1
2 The European Commission recommends a chronic Acceptable Daily Intake (ADI) of 0.024
3 mg/kg bw/day based on a chronic study in rats and a “Safety Factor” of 100 (European
4 Commission 2006, Appendix II, p.8). Note that ADIs and RfDs are functionally identical and
5 the term “Safety Factor” is used in the European literature as a functional synonym for the term
6 “Uncertainty Factor” used in most of the U.S. literature. This RfD is maintained in the most
7 recent review of spinosad by the European Food Safety Authority (EFSA 2013, p. 2). The study
8 on which the European ADI is based is not identified in European Commission (2006) or EFSA
9 (2011, 2012, 2013) documents. Based on the review of spinosad by WHO/FAO (2001, p. 58),
10 the chronic study in rats used by the European Commission (2006) appears to be the study by
11 Bond et al. (1995b MRIDs 43701507 and 43710503). As summarized in Appendix 1, Table A1-
12 2, the EPA evaluated this study and determined a NOAEL of 9.5 mg/kg bw/day (i.e., the dose for
13 males in the 200 ppm exposure group). FAO/WHO (2001) classifies 9.5 mg/kg bw as a LOAEL
14 and 2.4 mg/kg bw/day (i.e., the 50 ppm exposure group) as a NOAEL. As detailed in Appendix
15 1, Table A1-2, the FAO/WHO classification is consistent with the DER for this study from EPA.
16

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

27 **3.3.4. Dose-Severity Relationships**

28 Forest Service risk assessments sometimes consider dose-severity relationships to more fully
29 characterize potential risks in exposure scenarios where the doses exceed the RfD. For spinosad,
30 this consideration is relevant because some of the exposure scenarios for members of the general
31 public lead to estimated doses, particularly at the upper bounds of exposures, which substantially
32 exceed the RfDs (Section 3.4).
33

34 As summarized in Table 16, the ratios of the LOAEL to the corresponding NOAEL are about 2
35 for both the acute RfD [$9.73 \text{ mg/kg bw/day} \div 4.9 \text{ mg/kg bw/day} \approx 1.9857$] and the chronic RfD
36 [$5.36 \text{ mg/kg bw/day} \div 2.49 \text{ mg/kg bw/day} \approx 2.1526$]. While these ratios might not reflect dose-
37 severity responses in human populations, they are the most objective basis for assessing potential
38 concerns for exceedances in the RfDs.
39

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

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

3
4 For the chronic RfD, the potential impact of conservative uncertainty factors may be explored, if
5 not necessarily refined, based on the species-to-species relationships illustrated in Figure 5. As
6 discussed in Section 3.1.5, the chronic LOAELs for mice, rats, and dogs are well described by a
7 standard allometric function. Based on this relationship, the LOAEL for a 70 kg mammal would
8 be estimated at about 4.5 mg/kg bw/day. This cannot be directly compared to the chronic RfD
9 because the RfD includes factors of 10 for both animal-to-human extrapolation as well as
10 sensitive individuals within the population. The allometric relationship may account for the
11 factor of 10 used for animal-to-human extrapolation but not the factor for sensitive individuals.
12 Apply the factor of 10 for sensitive individuals, the estimated LOAEL for humans would be 0.45
13 mg/kg bw/day. As discussed in Section 3.3.3, the chronic RfD used in the current risk
14 assessment is 0.0249 mg/kg bw/day. Thus, the allometric relationship for the chronic toxicity of
15 spinosad suggests that a hazard quotient of about 18 [$0.45 \text{ mg/kg bw/day} \div 0.0249 \text{ mg/kg bw/day}$
16 ≈ 18.0722] would be viewed as an unacceptable exposure. Based on effects observed at the
17 LOAEL in the chronic study on dogs, adverse effects could include organ pathology; however,
18 overt toxic effects might not be observed.

19
20 Levels of exposure that might be associated with frank signs of toxicity cannot be clearly
21 determined based on the limited human data on spinosad (i.e., Su et al. 2011 as discussed in
22 Section 3.1.4.2) and low acute toxicity of spinosad by all routes of administration (Sections
23 3.1.4.1, 3.1.12, and 3.1.13). For the current risk assessment, these limitations in assessing dose-
24 severity relationships at exposures exceeding a hazard quotient of about 18 (discussed above) are
25 not a practical concern. As discussed further in Section 3.4, the highest hazard quotient in the
26 risk characterization is 15.

27

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

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

3 **3.4.2. Workers**

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

11
12 The benign risk characterization for workers is qualitatively similar to the risk
13 characterizations for workers given in EPA risk assessments. In the most recent completed
14 risk assessment for workers, the EPA maintains that . . . *risks [to workers] are not of concern*
15 (U.S. EPA/OPP/HED 2009a, p. 38). This language is also reflected in the EPA's scoping
16 document for the registration review of spinosad (U.S. EPA/OPP/HED 2011a, p. 22).

17
18 The only reservation in the risk characterization involves the potential for eye irritation. As
19 discussed in Section 3.1.11.3, U.S. EPA/OPP/HED (1997b, 2009a, 2010b, 2011a) categorizes
20 both technical grade spinosad and an unspecified 44% a.i. formulation of spinosad as Category
21 IV (i.e., the least severe category) for eye irritation. Nonetheless, as summarized in Table 4,
22 the Safety Data Sheet for the Entrust [80% a.i., WP] formulation is atypical in indicating that
23 the formulation . . . *Causes serious eye irritation*. In addition, the SDS for Entrust as well as the
24 SDS for Conserve SC (11.6% a.i.) and SpinTor 2SC (22.8% a.i.) indicate that the formulations
25 . . . *May cause pain disproportionate to the level of irritation to eye tissues*. While not required
26 on the product labels, the MSDS/SDS for the representative formulations considered in the
27 current risk assessment (Table 4) recommend the use of protective eyewear. Prudence suggests
28 that this cautionary language on the MSDS/SDS should be considered in any application of these
29 formulations.

30 **3.4.3. General Public**

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

39
40 At the central estimates, none of the HQs following a single application exceeds the level of
41 concern. For two applications, the HQ for the consumption of contaminated vegetation
42 (HQ=1.1) modestly exceeds the level of concern. At the upper bounds, the acute and chronic
43 HQs for the consumption of contaminated vegetation following a single application are identical
44 (HQ=6). Following two applications, the upper bound HQ for the consumption of contaminated
45 vegetation for acute exposure (HQ=12) is twice that for a single application. For longer-term

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

10
11 As discussed in Section 3.3.4, HQs above 2 would be associated with LOAELs in experimental
12 mammals based on the ratio of the LOAEL to the NOAEL. In other words, HQs in excess of 2
13 could raise concern for covert adverse effects. Based on allometric relationships for chronic
14 toxicity, HQs of up to 18 could be associated with covert adverse effects but not with signs of
15 frank toxicity. Levels of exposure that might be associated with overt adverse effects cannot be
16 identified. Based on these relationships, the modest exceedance (HQ=1.1) based on the central
17 estimate of exposure for the consumption of contaminated vegetation following two applications
18 does not raise substantial concern. While the upper bound HQs in the range of 1.6 to 12
19 associated with contaminated vegetation or fruit would probably not be associated with frank
20 signs of toxicity, the levels of exposure are in excess of exposures that would be considered
21 acceptable. If spinosad is sprayed on vegetation that might be consumed by humans, measures
22 to mitigate exposures to members of the general public would be prudent.

23
24 The accidental exposures associated with direct spray are below the level of concern (i.e., a
25 maximum HQ of 0.2). Accidental spills, however, lead to HQs of up to 15 (i.e., the consumption
26 of contaminated fish by subsistence populations). As with the non-accidental exposures, there is
27 no basis for asserting that accidental spills would lead to overt toxic effects in members of the
28 general public. Nonetheless, these HQs justify measures to reduce/mitigate exposures.

29 **3.4.4. Sensitive Subgroups**

30 For exposures to almost any chemical, there is particular concern for children, women who are
31 pregnant or may become pregnant, the elderly, or individuals with any number of different
32 diseases. Nonetheless, there are no reports in the literature suggesting subgroups that may be
33 unusually sensitive to spinosad. Under the Food Quality Protection Act (FQPA), the EPA is
34 required to consider populations that might be at increased risk to pesticide exposures including
35 considerations of reproductive effects, neurologic effects, and effects on immune function. Each
36 of these effects is considered in Section 3.1. Consistent with the current risk assessment, the
37 EPA determined that these endpoints do not justify quantitative changes in the dose-response
38 assessment (U.S. EPA/OPP/HED 2011a, p. 6).

39
40 Given the available information on spinosad, subgroups in the human population that might be
41 atypically sensitive to spinosad have not been identified.

42 **3.4.5. Connected Actions**

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

1 Actions are considered to be connected if they: (i) Automatically trigger other actions which may
2 require environmental impact statements; (ii) Cannot or will not proceed unless other actions are
3 taken previously or simultaneously, and (iii) Are interdependent parts of a larger action and
4 depend on the larger action for their justification. Within the context of this assessment of
5 spinosad, “connected actions” include actions or the use of other chemicals which are necessary
6 and occur in close association with use of spinosad.

7
8 Spinosad formulations contain inert components, and the metabolism of spinosad may involve
9 the formation of a number of different compounds. Thus, spinosad applications will entail
10 (automatically trigger) exposures to inerts as well as metabolites. As discussed in detail in
11 Sections 3.1.14 (Inerts and Adjuvants), the disclosed inerts in spinosad formulations do not
12 appear to present hazards that require quantitative consideration. As discussed in Section 3.1.15
13 (Impurities and Metabolites) and implemented in the exposure assessments (Sections 3.2 and
14 4.2), the metabolites of spinosad are explicitly considered using the total residue approach
15 similar to that employed in U.S. EPA/OPP/HED (2011a, p. 9).

16 **3.4.6. Cumulative Effects**

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

20
21 The U.S. EPA/OPP makes the following assessment of cumulative risk for both spinosad and
22 spinetoram:

23
24 *Unlike other pesticides for which EPA has followed a cumulative risk*
25 *approach based on a common mechanism of toxicity, EPA has not made a*
26 *common mechanism of toxicity finding as to spinetoram/spinosad and any*
27 *other substance and spinetoram/spinosad do not appear to produce a toxic*
28 *metabolite produced by other substances. For the purposes of this*
29 *tolerance action, therefore, EPA has not assumed that spinetoram/*
30 *spinosad does not have a common mechanism of toxicity with other*
31 *substances.*

32 U.S. EPA/OPP (2009a, pp. 32)

33
34 The human health risk assessment scoping document contains similar language and indicates that
35 the EPA will review any new information relating to potential cumulative risks with other
36 pesticides (U.S. EPA/OPP/HED 2011a).

37
38 Explicit in the above determination, as discussed in Section 3.1.5, the EPA has determined that
39 spinosad and spinetoram have a common mechanism of action and are toxicologically
40 equivalent. Thus, if spinosad and spinetoram are used concurrently in the same location, the
41 cumulative effects of both spinosad and spinetoram should be considered quantitatively.

42
43 As discussed in Section 3.1.16 (Toxicological Interactions), spinosad may enhance the toxicity
44 of other compounds (e.g., ivermectin and milbemycin oxime), possibly via an inhibition of
45 P-glycoprotein or competition with cytochrome P450. P-glycoprotein and cytochrome P450 play
46 significant roles in the metabolism and/or elimination of a wide-variety of compounds, both

1 naturally occurring and synthetic. The occurrence and nature of any interactions will depend on
2 the levels of exposure as well as the specific mechanism(s) for any interactions between spinosad
3 and the other compounds. Further generalizations are not warranted by the available
4 information.
5

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 LD₅₀ values in insects ranging from about 0.025 to 65 mg/kg bw versus LD₅₀ 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 LD₅₀ values are available only on mice and rats; furthermore, most of the LD₅₀ values are indefinite—i.e., expressed as greater than a given dose. Definitive LD₅₀ 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.

1
2 While the available data are not compelling, dogs and other canids are identified as a subgroup
3 of mammals that may be more sensitive than rodents to spinosad exposure. This issue is
4 addressed further in the dose-response assessment for mammals. Given the limitations in the
5 allometric relationships in the chronic LOAEL studies and the lack of an allometric relationship
6 in the subchronic LOAEL studies, separate toxicity values are not derived for small and large
7 non-canid mammals.

8 **4.1.2.2. Birds**

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

13
14 The acute gavage studies in birds typically involve the administration of single doses with a 14-
15 day observation period (U.S. EPA/OCSP 2012a). The gavage studies in mallards (Murray
16 1992, MRID 43414528) and quail (Murray et al. 1992b, MRID 43414529) are somewhat
17 atypical in that three doses were administered over a 6-hour period. As noted in the DERs for
18 these studies, the full studies submitted to EPA do not provide a rationale for the multiple doses.
19 As detailed in Appendix 2, Table A2-1, the total doses administered to the birds were 0, 200,
20 500, 1000, or 2000 mg/kg bw, none of which caused mortality—i.e., the LD₅₀ could be specified
21 as >2000 mg/kg bw, which is how the LD₅₀ values are specified for mallards and quail in the
22 review by the European Commission (2006, p. 24). This characterization of the LD₅₀ would
23 result in a classification of spinosad as Practically Nontoxic (e.g., SERA 2014a, Table 16).
24 Because the doses were spaced over a 6-hour period, however, the EPA designates the maximum
25 dose at 1333 mg/kg bw (2/3 x 2000) and classifies spinosad as *Slightly Toxic* (U.S.
26 EPA/OPP/EFED 2011a, p. 35). While mortality was not observed in either study, quail
27 evidenced signs of toxicity at all but the lowest dose (i.e., NOAEL = 200 mg/kg bw). No signs
28 of toxicity were observed in mallards at doses up to 2000 mg/kg bw. Thus, quail appear to be
29 more sensitive than mallards to spinosad. As discussed in Section 3.1.6, spinosad does not
30 appear to be neurotoxic in mammals. While the study in quail does note ataxia in quail at doses
31 of 500 mg/kg bw and above, it is not clear if the ataxia can be regarded as a direct neurotoxic
32 effect. Nonetheless, in the absence of other signs of toxicity, the occurrence of ataxia is
33 suggestive of neurotoxicity in quail.

34
35 The acute dietary studies in mallards (Murray and Woolwine 1992, MRID 43414530) and quail
36 (Murray et al. 1992a, MRID 43414531) are similar to the acute gavage studies in that the
37 reported LC₅₀ values are indefinite, specifically >5156 ppm for both mallards and quail. Based
38 on the LC₅₀ values, the EPA classifies spinosad as *Practically Nontoxic* to birds in terms of acute
39 dietary exposures (U.S. EPA/OPP/EFED 2011a, p. 36). Also as with the acute gavage studies,
40 quail appear to be more sensitive than mallards to spinosad. As detailed in Appendix 2, Table
41 A2-2, there was no mortality or signs of toxicity in mallards exposed to dietary concentrations of
42 up to 5156 ppm. In quail, signs of toxicity included decreased body weight at concentrations of
43 1335 ppm and above, loose feces at concentrations of 5253 ppm, and mortality (1/10) at
44 concentrations of 2601 and 5252 ppm. The NOAEL for quail was 656 ppm. As detailed in
45 Appendix 2, Table A2-2, the NOAEL of 656 ppm corresponds to a dose of about 200 mg/kg
46 bw/day based on approximate food consumption rates from similar studies on other pesticides

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

4
5 Unlike the acute toxicity studies, there are no remarkable sensitivity differences among quail and
6 mallards demonstrated in the available reproduction studies (Appendix 2, Table A2-3). In both
7 species, adverse reproductive effects were noted at 1100 ppm. The reproductive effects were
8 severe and characterized by decreases in live embryos and offspring survival in both species.
9 Also in both species, no adverse effects on adults, offspring, or reproductive parameters were
10 noted at 550 ppm. The DERs for the study in quail (Beavers et al. 1994a, MRID 43414533) and
11 the study in mallards (Beavers et al. 1994b, MRID 43414532) do not provide sufficient
12 information to estimate doses in units of mg/kg bw/day. For both mallards and quail, dietary
13 concentrations (mg/kg diet) are converted to mg/kg bw/day doses using a food consumption
14 factor of 0.07 kg food/kg bw based on reproduction studies in quail and mallards (SERA 2007b).
15 Based on this food consumption factor, the dietary NOAEC of 550 mg a.i./kg diet corresponds to
16 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]
17 and the LOAEC corresponds to a dose of about 77 mg/kg bw/day [1100 mg/kg food x 0.07 kg
18 food/kg bw = 77 mg/kg bw]. As discussed in Section 3.1.9.2, the LOAEL in birds is similar to
19 the LOAEL of 100 mg/kg bw/day in rats (Breslin et al. 1994, MRIDs 43701506) which is based
20 on comparable endpoints—i.e., decreases in litter size and offspring survival. The NOAEL in
21 mammals (10 mg/kg bw/day) is lower than the estimated LOAEL in birds (38.5 mg/kg bw/day);
22 nevertheless, this difference may be an artifact of the dose spacing in the studies rather than a
23 true difference in sensitivity between mammals and birds.

24
25 The avian toxicity studies in the open literature on spinosad do not substantially expand the
26 information directly useful in the hazard identification for birds. In an abstract of a residue
27 feeding study in hens, Magnussen et al. (1996) note that spinosad accumulates primarily in the
28 liver and fat with metabolites reflecting N-demethylation or O-demethylation. This general
29 pattern is similar to that in mammals (Section 3.1.3.1). Spinosad is used in poultry production for
30 the control of the poultry red mite, *Dermanyssus gallinae*. In this use, spinosad solutions in the
31 range of 2000 to 4000 mg a.i./L are sprayed in nesting facilities. Studies documenting the
32 efficacy of this use (e.g., George et al. 2010; Leibisch et al. 2011) do not indicate adverse effects
33 on hens or egg production. Given the nature of the exposures, estimates of doses, in units of
34 mg/kg bw, to the chickens in treated facilities cannot be made. Uggini et al. (2012) examined the
35 effect of spinosad solutions on chicken eggs via direct injection. While adverse effects were not
36 noted at spinosad concentrations of 100 µg/egg, deformities (skull, sternum, and ribcage) were
37 noted at doses of 500 and 750 µg/egg. Again, these estimates of exposure are not directly
38 comparable to the data from the reproduction studies in birds (discussed above).

39 **4.1.2.3. Reptiles and Amphibians (Terrestrial-Phase)**

40 There are no data regarding the toxicity of spinosad to reptiles or terrestrial-phase amphibians in
41 the EPA or APHIS ecological risk assessments (U.S. EPA/OPP/EFED 2005, 2009a, 2010a,
42 2011a) and (USDA/APHIS 2003, 2011, 2014), or in the review by Pauli et al. (2000). No other
43 information on the toxicity of spinosad to reptiles or terrestrial-phase amphibians was identified
44 in the open literature. As noted in the EPA risk assessments, the EPA recommends the use of
45 birds as surrogates for reptiles and terrestrial-phase amphibians.

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

8 **4.1.2.4. Terrestrial Invertebrates**

9 **4.1.2.4.1. General Considerations**

10 As discussed in SERA (2014a, Section 4.1.2.4), assays for toxicity to the honeybee are standard
11 EPA requirements for pesticide registration, and acute toxicity data on the honeybee involving
12 oral and contact assays are commonly used as a surrogate for other terrestrial invertebrates. As
13 discussed further below, the literature on the effects of spinosad on terrestrial insects is extensive
14 and covers many different species. Nonetheless, the effects of spinosad on bees are important to
15 the assessment of the potential effects of spinosad on pollinators. All of the product labels for
16 the formulations of spinosad specifically encompassed by the current risk assessment (Table 3)
17 contain relatively standard language on potential effects to pollinators:
18

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

23 Product label for Entrust SC

24
25 The genesis of the “3 hour” language is discussed further in Section 4.1.2.4.4.2 (Field and Field
26 Simulation Studies).

27
28 In terms of practical utility to the risk assessment, the most relevant studies are those for which
29 defined doses in units of mg/kg bw can be determined. These studies are addressed in Section
30 4.1.2.4.2 (Oral Toxicity) and Section 4.1.2.4.3 (Contact Toxicity). A limitation in these studies
31 is that only relatively few species of terrestrial invertebrates have been assayed. This limitation
32 is addressed with analyses of other toxicity studies using the Organization for Biological and
33 Integrated Control of Noxious Animals and Plants (IOBC) system (Section 4.1.2.4.4), efficacy
34 studies from ECOTOX (Section 4.1.2.4.5), and considerations of resistance in insect populations
35 (Section 4.1.2.4.6). While most of the toxicity data on terrestrial invertebrates involve studies on
36 insects and other arthropods, the limited information on earthworms is addressed in Section
37 4.1.2.4.7.

1
2 Mechanistically, spinosyns act on the insect nervous system causing excitation of the neurons,
3 primarily by the stimulation of nicotinic acetylcholine (nAChR) receptors and secondarily by the
4 stimulation of gamma-aminobutyric acid (GABA) gated chloride channels (Barbosa et al. 2015;
5 Shi et al. 2011; Thompson et al. 2000, 2015; U.S. EPA/OPP 2009a, 2012a; U.S.
6 EPA/OPP/EFED 2005, 2011a). In terms of resistance, the spinosyns, including spinosad, are
7 classified as Group 5 nAChR modulators but are considered distinct from other types of nAChR
8 modulators in Group 4 which includes the neonicotinoids (IARC 2015). In worker honeybees,
9 spinosad is associated with both the inhibition of both AChE and ATPase—i.e., an enzyme
10 central to energy metabolism (Rabea et al. 2010). These biochemical mechanisms are associated
11 with gross signs of neurotoxicity that include tremors and involuntary muscle contractions which
12 can lead to neuromuscular fatigue, paralysis, cessation of feeding, and eventually death (e.g.,
13 Musser and Shelton 2005; Salgado 1998, Salgado et al. 1998; Thompson et al. 2000).

14
15 The sublethal effects of spinosad include impaired flight (Tom et al. 2015) as well as
16 reproductive impairment which is demonstrated in several groups of insects including
17 Lepidoptera (Pineda et al. 2007), Neuroptera (Rimoldi et al. 2012), Diptera (Romi et al. 2006),
18 and Hymenoptera (Schneider et al. 2004; Wang et al. 2012a).

19
20 Thompson et al. (2015) note that spinosad ...*demonstrates rapid contact and ingestion activity in*
21 *insects which is unusual for a biological product*. This statement appears to refer to sublethal
22 rather than lethal effects. In terms of lethality, insect death may not occur until several days after
23 initial exposure (Nowak et al. 2001; Thompson et al. 2000). As discussed below and detailed in
24 Appendix 3, marked temporal effects are apparent in some studies on acute toxicity (e.g., Herzog
25 et al. 2002; Schneider et al. 2004).

26
27 For most insecticides, toxicity tends to increase with increasing temperatures, and this pattern
28 was observed in grasshoppers (Amarasekare and Edelson 2004). The opposite pattern—i.e.,
29 decreasing toxicity with increasing temperature—was observed in houseflies (Diptera, Khan and
30 Akram 2014) and corn borers (Lepidoptera, Musser and Shelton 2005). The available data do
31 not address the effect of temperature on spinosad toxicity to bees.

32
33 While not explicitly covered in the current risk assessment, spinosad baits, specifically GF120,
34 are used for the control of fruit flies. Several studies note the avoidance of these bait
35 formulations by some bees, including honeybees (Cabrera-Marin et al. 2015; Gomez-Escobar et
36 al. 2014; Mangan and Moreno 2009), as well as other groups of nontarget insects (Cisneros et al.
37 2002; Michaud 2003). The avoidance of spinosad baits by bumble bees (Morandin et al. 2005),
38 *Plebeia moureana* (Hymenoptera: Apidae), a species of stingless bee (Sanchez et al. 2012), or
39 some nontarget dipterans (Wang and Messing 2006) is not documented in the available literature.
40 Apart from the GF120 bait formulation, no avoidance of a 480 g/L formulation (i.e., Tracer[®])
41 was noted in a controlled laboratory study with *Chelonus insularis*, a nontarget hymenopteran
42 parasitoid.

43
44 As discussed further in Section 4.1.2.4.6, several studies address the development of insect
45 resistance to spinosad. Many of these studies note that piperonyl butoxide and other inhibitors of
46 cytochrome P450 synergize the toxicity of spinosad (Bao et al. 2014; Markussen and Kristensen

1 2012; Scott 1998). The synergism of insecticides by inhibitors of cytochrome P450 suggests that
2 at least some insects metabolize spinosad via cytochrome P450 and that this metabolism is a
3 detoxification pathway.

4 **4.1.2.4.2. Oral Toxicity**

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

15
16 Oral LD₅₀ values are available only for bees [Hymenoptera] from the Apidae family—i.e., seven
17 LD₅₀ values for the honeybee (*Apis mellifera*) and one LD₅₀ value each for a bumblebee
18 (*Bombus terrestris*) and a stingless bee (*Melipona quadrifasciata*). The oral LD₅₀ values in
19 honeybees are remarkably consistent with a range of 0.41 to 0.52 mg a.i./kg bw. Based on the
20 review by the European Commission (2006) the toxicity of the NAF-85 formulation (i.e., LD₅₀ =
21 0.42 mg a.i./kg) is not remarkably different from the toxicity of technical grade spinosad. As
22 noted in Table 1, NAF-85 is a 44.2% a.i. formulation. Similarly, Mayes et al. (2003) reports an
23 LD₅₀ for the honeybee of 0.50 mg a.i./kg bw for a 480 SC formulation. The similarities between
24 the toxicity values for technical grade spinosad and spinosad formulations suggest the inerts in
25 the formulations do not have a substantial impact of the toxicity of the formulations to
26 honeybees.

27
28 Based on assays using the 480 SC formulation of spinosad, the honeybee appears to be more
29 sensitive by a factor of about 3, compared with either the bumble bee [$0.50 \div 0.13 \approx 3.84$] or the
30 stingless bee (*Melipona quadrifasciata*) [$0.50 \div 0.15 \approx 3.33$]. As discussed further in Section
31 4.1.2.4.3 (Contact Toxicity), the apparently greater sensitivity of the bumble bee relative to the
32 honeybee following oral exposure is not reflected in the contact toxicity studies.

33
34 In addition to the acute oral toxicity studies on adult bees, discussed above, Barbosa et al. (2015)
35 conducted a 20-day oral toxicity study on larvae of another species of stingless bee, *Melipona*
36 *quadrifasciata*. This study also used a 480 g a.i./L SC formulation of spinosad. As summarized
37 in Appendix 3, Table A3-2, signs of toxicity in larvae included decreased survival, decreased
38 pupal body weights, and increases in the incidence of malformations at doses of about 0.059 mg
39 a.i./kg bw/day and higher. No signs of larval toxicity were observed at or below doses of 0.012
40 mg a.i./kg bw/day. In newly emerged adults, however, abnormal walking activity was observed
41 at doses of about 0.0012 mg a.i./kg bw/day or higher. The NOAEL for changes in walking
42 activity was about 0.00059 mg a.i./kg bw/day. The publication does not provide an estimate of
43 the LD₅₀ but does provide a survival probability plot (Barbosa et al. 2015, Figure 1B). Based on
44 this plot, 50% mortality occurred at about 18 days after dosing and at reported doses of about
45 0.059 to 0.12 mg a.i./kg bw/day. As discussed above and summarized in Table 17, these doses

1 are only moderately below the acute oral LD₅₀ of 0.15 mg a.i./kg bw for *Melipona quadrifasciata*
2 (Tom et al. 2015).

3
4 In addition to the studies on bees, Table 17 also includes LD₅₀ values for the American
5 cockroach (*Periplaneta americana*) and the tobacco budworm (*Heliothis virescens*) larvae from
6 the study by Salgado (1998) following abdominal injection of spinosyn A. As discussed in
7 Section 2, spinosyn A is the major component of spinosad. While these data are not directly
8 comparable to oral toxicity data, the relatively high LD₅₀ values in the cockroach (i.e., 1.1 and
9 2.7 mg/kg bw) suggest that cockroaches may be somewhat less sensitive than bees. The
10 relatively low LD₅₀ value of 0.23 mg/kg bw in tobacco budworm larvae is intermediate between
11 the low oral LD₅₀ values for the bumblebee and stingless bee and the somewhat higher LD₅₀
12 values for honeybees.

13 **4.1.2.4.3. Contact Toxicity**

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

21 **4.1.2.4.3.1. Contact Toxicity to Bees**

22
23 Based on the contact LD₅₀ of 2.9 ng (≈0.025 mg/kg bw) from Hoxter et al. (1992), the EPA
24 classifies technical grade spinosad as ...*highly toxic toward honey bees* (U.S. EPA/OPP/EFED
25 2011a, p. 35). As summarized in Table 19, however, the variability in the toxicity data for
26 technical grade spinosad in *Apis mellifera* is much greater than the corresponding values for oral
27 toxicity—i.e., LD₅₀ values of about 0.5 mg/kg bw (Section 4.1.2.4.1). The relatively low LD₅₀ of
28 0.025 mg/kg bw from Hoxter et al. (1992) is well documented and is supported by an LD₅₀ of
29 about 0.031 mg/kg bw from the European Commission (2006). Details of the study used by the
30 European Commission (2006) are not available. Three other contact LD₅₀ values for technical
31 grade spinosad in *Apis mellifera* range from 0.34 to 0.67 mg/kg bw and are much closer to the
32 oral LD₅₀ values. As discussed further in Section 4.1.2.4.6 (Resistance), differences in
33 sensitivity of about a factor of 10 might be expected from organisms taken from different
34 populations in the field. Whether or not this might account for the differences in the LD₅₀ values
35 for technical grade spinosad in *Apis mellifera* cannot be determined from the available data.

36
37 Another difference between the oral and contact LD₅₀ values for *Apis mellifera* involves the
38 toxicity of formulations. As discussed in Section 4.1.2.4.2 and summarized in Table 17, the oral
39 LD₅₀ values for technical grade spinosad are similar to the LD₅₀ values for formulations. Based
40 on the LD₅₀ values for topical applications, a 1.6% wettable power (WP) formulation appears to
41 be comparable in toxicity to technical grade spinosad, and the 480 suspension concentrate (SC)
42 formulation (i.e., 480 g/L) appears to be substantially less toxic than technical grade spinosad.
43 Consistent with data in mammals (Section 3.1.14.1), the data for the honeybees do not suggest
44 that other ingredients (i.e., inerts) in spinosad formulations contribute substantially to toxicity.
45

1 In addition to studies on honeybees, data are available on other bee species including the bumble
2 bee (*Bombus terrestris*, Apidae), the alfalfa leafcutter bee (*Megachile rotundata*, Megachilidae),
3 and the alkali bee (*Nomia melanderi*, Halictidae). The study by Mayer et al. (2001) provides
4 data on the latter two species as well as the honeybee, and these may be considered essentially
5 matched bioassays—i.e., conducted using the same methods by the same set of investigators.
6 Based on the data from Mayer et al. (2001), the honeybee appears to be the most sensitive bee
7 species with an LD₅₀ of 0.61 mg/kg bw. The alkali bee (*Nomia melanderi*, Halictidae) is
8 somewhat less sensitive with an LD₅₀ of 0.76 mg/kg bw. The alfalfa leafcutter bee (*Megachile*
9 *rotundata*, Megachilidae), with an LD₅₀ of 1.9 mg/kg bw, appears to be less sensitive than the
10 honey bee by a factor of about 3 [1.9 mg/kg bw ÷ 0.61 mg/kg bw ≈ 3.1148]. As specified in
11 Appendix 3, however, the 95% confidence intervals for the three LD₅₀ values overlap – i.e., the
12 apparent differences in sensitivities may not be statistically significant.

13
14 The data on the bumble bee come from the review by Mayes et al. (2003) summarizing a study
15 conducted by Dow AgroSciences. As discussed in Section 4.1.2.4.2 (oral toxicity) and
16 summarized in Table 17, the bumble bee appears to be more sensitive than the honey bee by
17 about a factor of 3 based on oral LD₅₀ values. Based on contact assays with a 480 SC
18 formulation, however, bumble bees appear to be substantially less sensitive than honey bees.
19 Taking the highest LD₅₀ for an 480 SC formulation, the magnitude of the difference in sensitivity
20 is about a factor of 8 [65 mg/kg bw (bumble bee) ÷ 8.5 mg/kg bw (honeybee) ≈ 7.6].

21
22 While the discussion of relative sensitivities focuses on doses that can be expressed in units of
23 mg/kg bw, the studies by Bailey et al. (2005) and Scott-Dupree et al. (2009) on direct spray
24 applications (i.e., LC₅₀ values expressed in units of mg/L) can be used to elaborate on differences
25 in sensitivities among bees. The papers by Bailey et al. (2005) and Scott-Dupree et al. (2009) are
26 from the same group of investigators using the same direct spray exposures. These two studies
27 are summarized in Table 20. Consistent with the standard micropipette studies discussed above,
28 the bumble bee appears to be less sensitive than the honeybee by a factor of about 4 [89.5 mg/L
29 (bumblebee) ÷ 22 mg/L (honeybee) ≈ 4.07]. Unlike the case with the topical applications from
30 Mayer et al. (2001) in which the leafcutter bee was less sensitive than the honeybee by a factor
31 of about 3 [1.9 mg/kg bw ÷ 0.61 mg/kg bw ≈ 3.1148], the direct spray assay from Scott-Dupree
32 et al. (2009) suggests that the leafcutter bee is more sensitive than the honeybee by a factor of
33 about 2 [12.5 mg/L (leafcutter bee) ÷ 22 mg/L (honeybee) ≈ 0.56; 1 ÷ 0.56 ≈ 1.78].

34 35 **4.1.2.4.3.2. Contact Toxicity to Other Terrestrial Insects**

36 Differences in toxicity among various groups of organisms are a fundamental concern in any
37 ecological risk assessment. In some cases, differences among species may systematically relate
38 to body weight even when dose is scaled to units of mg/kg bw (i.e., allometric relationships as
39 discussed by Sample and Arenal 1999). Based on the contact LD₅₀ values (in units of mg/kg bw)
40 from Table 19 and as illustrated in Figure 6, this does not appear to be the case for differences in
41 the toxicity of spinosad in insects. Over a relatively wide range of body weights (i.e., about 2 mg
42 to 30 mg), no systematic differences in sensitivity are apparent for three orders of Diptera,
43 including Muscidae (house flies), Culicidae (mosquitoes), and Tephritidae (fruit flies). As
44 discussed in Section 4.1.2.4.3.1, bumblebees [Hymenoptera: Aphidae, tribe Bombini] appear to
45 be more tolerant than other hymenopterans to spinosad. No other substantial trends in toxicity
46 are apparent for the other hymenopterans over a range of body weights that exceeds an order of

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

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

$$18 \quad \text{Freq}_i = \frac{i - 0.5}{N} \quad (5)$$

19
20 where Freq_i is the cumulative frequency for the i^{th} value and N is the number of values in the
21 data set. As detailed by Posthuma et al. (2002), the development of sensitivity distributions
22 involves an ordered ranking of the available toxicity values (i.e., lowest to highest) in which “ i ”
23 in the above equation is the ordinal rank – i.e., 1st, 2nd, 3rd, and so on. The 0.5 constant in the
24 above equation is factor to adjust for the ordinal ranking to approximate a midpoint. For
25 example, thirteen LD₅₀ values are available for the hymenopterans (Table 18). The lowest value
26 is 0.025 mg/kg bw, the LD₅₀ for *Aphis mellifera* from the study by Hoxter et al. (1992). The
27 frequency for this value is about 0.038462 [(1-0.5) ÷ 13]. The second lowest LD₅₀ for
28 hymenopterans is 0.031 mg/kg bw, also for *Aphis mellifera* (European Commission 2006). This
29 frequency is about 0.115385 [(2-0.5) ÷ 13]. The x-axis in Figure 7 represents the LD₅₀ value
30 corresponding to the frequency. The x-axis uses a logarithmic scale under the standard
31 assumption that LD₅₀ values for different chemicals or different groups of organisms have a
32 lognormal distribution.

33
34
35 As illustrated in Figure 7, no remarkable differences in sensitivity are apparent among the three
36 orders of insects for the left-most points (i.e., areas of greater sensitivity). The data on
37 Lepidoptera are limited to only two points. Nonetheless, the similarities among the
38 Hymenoptera, Diptera, and Lepidoptera are striking in terms of similarities among presumably
39 sensitive species in these orders of insects.

40
41 The upper and right-most three points in Figure 7 for the Hymenoptera, however, appear to be
42 somewhat right-shifted in that these points appear to reflect an atypical tolerance to spinosad.
43 This is particularly true for the right-most point which is for the bumblebee (*Bombus terrestris*,
44 Apidae). This point is from the study by Mayes et al. (2003) using a 480 SC formulation. As
45 discussed in Section 4.1.2.4.3.1, the bumblebee appears to be substantially more tolerant to this

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

9 **4.1.2.4.4. Other Toxicity Studies**

10 **4.1.2.4.4.1. IOBC Classifications**

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

$$25 \quad S = \sum_{i=1}^4 i \times \frac{n_i}{N} \quad (6)$$

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

35
36 Parasitic wasps (Hymenoptera) are rated as the most sensitive group of terrestrial insects by both
37 Williams et al. (2003b) and Miles and Eelen (2006). This designation is consistent with the more
38 recent review by Biondi et al. (2012) as well as several other studies on the sensitivity of
39 hymenopteran wasps and other hymenopteran parasitoids (e.g., Beloti et al. 2015; Biondi et al.
40 2013; de Freitas Bueno et al. 2008; Liu and Zhang 2012). In addition to acute lethality, as
41 discussed in Section 4.1.2.4.1, the sublethal toxicity of spinosad involves adverse effects on
42 reproduction, and several studies on Hymenoptera demonstrate adverse effects on reproductive
43 parameters and longevity (Beloti et al. 2015; Liu and Zhang 2012; Penagos et al. 2005;
44 Schneider et al. 2004; Wang et al. 2012a). A few matched studies involving multiple orders of

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

4
5 The only inconsistency between the rankings from Williams et al. (2003b) and Miles and Eelen
6 (2006) involves the arthropods in the subclass Acari (mites and ticks). This inconsistency is
7 relatively trivial given that Williams et al. (2003b) covered only four studies on the Acari and
8 the more recent analysis by Miles and Eelen (2006) covered 40 studies. The weight-of-evidence
9 suggests that some mites may be highly sensitive (e.g., *Neoseiulus fallacis* in the study by
10 Villanueva and Walgenbach 2005) but that most mites are less sensitive than wasps to spinosad.
11 The only other groups that appear to be highly sensitive to spinosad are the Dermoptera
12 (earwigs) and Thysanoptera (thrips). As with the Acari in the analysis by Williams et al.
13 (2003b), however, the high composite scores are based on only a few studies, including three
14 studies on Dermoptera in the analysis by Williams et al. (2003b) and two studies on
15 Thysanoptera in the analysis by Miles and Eelen (2006). Nonetheless, as discussed further in
16 Section 4.1.2.4.7 (resistance), spinosad is used extensively for the control of thrips (U.S.
17 EPA/OPP/EFED 2011a), and the apparent high sensitivity of thrips to spinosad is probably not
18 an artifact of small sample size. While the effects of spinosad on Dermoptera are not extensively
19 documented, Cisneros et al. (2002) and Redoan et al. (2013) note that spinosad is detrimental to
20 *Doru* species (Dermoptera: Forficulidae), predators on lepidopteran pests. Alston and Tebeau
21 (2011) recommend spinosad (specifically the Success and Entrust formulations) in discussing
22 methods for the control of the European earwig (*Forficula auricularia*, Dermoptera:
23 Forficulidae), which may be viewed as a pest species on some crops. While this
24 recommendation reinforces the assessment that Dermoptera may be an insect order sensitive to
25 spinosad, the specimen product labels for Entrust SC[®] and Success[®] are not specifically labelled
26 for the control of earwigs.

27
28 The application of the IOBC system by Williams et al. (2003b) and Miles and Eelen (2006) do
29 not specifically address bees (several orders of Hymenoptera) and Lepidoptera. Field studies
30 with bees are discussed in the following subsection. As discussed in Section 4.1.2.4.5, spinosad
31 is highly toxic to and used to control lepidopteran pests, as indicated by the large number of
32 efficacy studies.

33 34 **4.1.2.4.4.2. Field and Field Simulation Studies (Bees)**

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

1
2 The study by Sanchez et al. (2012) is a field simulation study involving extremely high
3 concentrations of spinosad in sucrose (i.e., 10 to 80 mg a.i./L) in assays of a stingless bee,
4 *Plebeia moureana*. As discussed further in Section 4.2.3.3 (Nectar Exposures Involving
5 Honeybees), nectar concentrations of 10 to 80 mg a.i./L are far greater than concentrations of
6 spinosad in nectar that might be expected in Forest Service applications. Sanchez et al. (2012)
7 indicate that there was no adverse effect on *Plebeia moureana*. As discussed in Section 4.1.2.4.2
8 and summarized in Table 17, another species of stingless bee (*Melipona quadrifasciata*) appears
9 to be somewhat more sensitive than the honeybee is to spinosad. While not explicitly assessing
10 the sensitivity of *Plebeia moureana* to spinosad, the study by Sanchez et al. (2012) suggests that
11 *Plebeia moureana* may be relatively insensitive to spinosad.
12

13 The open literature study by Burns et al. (2001) used low application rates of up to about 0.0014
14 lb a.i./acre in efficacy tests for fruit fly control. No adverse effects were observed in foraging
15 bees. This study is considered below in conjunction with several other low application rate
16 studies noting no adverse effects in bees.
17

18 The open literature study by Morandin et al. (2005) is a field simulation study in which
19 bumblebee (*Bombus impatiens*) colonies were treated with spinosad-contaminated pollen. While
20 exposures are expressed as concentrations of spinosad in pollen (in units of mg a.i./kg pollen),
21 Morandin et al. (2005) provide estimates of application rates that might be associated with the
22 concentrations of pollen used in the study. Despite some uncertainty with the usefulness of this
23 study, the observations by Morandin et al. (2005) are discussed below with other field or field
24 simulation studies summarized in Mayes et al. (2003).
25

26 An overview of the field and field simulation studies is given in Table 22. Most of the studies
27 summarized in Appendix 3, Table A3-4 express exposure in units of g a.i./ha. In Table A3-4,
28 units in lb a.i./acre are given in brackets [] using the conversion factor of $0.892 \frac{\text{lb/acre}}{\text{kg/ha}}$ with
29 rounding to two significant figures. In Table 22, only units of lb a.i./acre are used. Note that
30 Table 22 does not include three greenhouse studies which are summarized at the start of
31 Appendix 3, Table A3-4. All three greenhouse studies are unpublished reports summarized in
32 Mayes et al. (2003). The two studies attributed to Kaneshi (200a,b) note adverse effects in
33 honeybees and bumblebees at relatively low application rates of ≈ 0.089 lb a.i./acre for honeybees
34 and 0.11 lb a.i./acre for bumblebees.
35

36 The greenhouse studies are not included in Table 22 because they are not typical of exposures
37 that would be used in Forest Service programs. Furthermore, as summarized in Table 22, many
38 field and field simulation studies indicate that adverse effects are not likely to occur in bees at
39 application rates of up to 0.16 lb a.i./acre. The specific studies include Burns et al. (2001) as
40 well as the following studies cited by Mayes et al. 2003 are: Forey 1999, Kirkland 1999 (low
41 application rate), Kransfelder 1999, Mayer 1999, Palmer and Krueger 1997, Taylor and Goodwin
42 2000, and Vinall 2000.
43

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

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

5
6 While contact toxicity (4.1.2.4.3.1) is used directly in the current risk assessment to characterize
7 the risks associated with direct spray, residual contact toxicity is a concern—i.e., the contact of a
8 bee with contaminated vegetation following the foliar application of spinosad. As noted in
9 Section 4.1.2.4.1, the product labels specify that spinosad applications may be ... *toxic to bees*
10 *exposed to treatment for 3 hours following treatment*. This language appears to reflect field
11 simulation studies (summarized in Appendix 3, Table A3-4) in which no signs of acute toxicity
12 were observed in bees following exposure to vegetation that was treated 3 hours prior to
13 exposing the bees to the vegetation (Mayes et al. 2003 citing unpublished studies by Kransfelder
14 1999; Palmer and Krueger 1997) and the lack of effects on mortality or brood development in
15 honeybee colonies following exposure to treated vegetation that had been covered for 3 hours—
16 i.e., effectively preventing direct exposure to the contaminated vegetation prior to drying of the
17 applied solution (Mayes et al. 2003 citing unpublished study by Mayer 1999). The reduced
18 toxicity to bees (i.e., no signs of overt toxicity) was also observed following applications of
19 spinosad conducted in the evening when bees are not actively foraging—i.e., Mayes et al. (2003)
20 citing unpublished studies by Taylor and Goodwin (2000) and Goodwin and Haine (1998).
21 Lastly, Mayer et al. (2001) conducted residual contact assays in alfalfa leafcutter bees and alkali
22 bees noted generally lower mortality using vegetation assayed at 8 hours after treatment relative
23 to 2 hour after treatment.

24
25 These field and field simulation studies are consistent with studies summarized in US
26 EPA/OPP/EFED (2011a) indicating that the toxicity of spinosad residues on vegetation is
27 substantially reduced by a post-application period of 3 hours prior to exposing bees to the
28 vegetation (i.e., MRID 45007701 and MRID 45007702 as summarized in Appendix 3, Table A3-
29 1). The impact of reducing exposures to bees during or for a period of time after application is
30 an important consideration in the risk characterization for bees (Section 4.4.2.4.1).

31 **4.1.2.4.5. Efficacy Studies**

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

1 Because of the small number of points for non-lepidopteran orders of insects, sensitivities
2 relative to lepidopterans can be assessed only crudely. Nonetheless, and consistent with the
3 sensitivity distributions for contact LD₅₀ values, the sensitivity of Lepidoptera appears to be
4 similar to that of Hymenoptera at least at the lower and upper bounds of the application rates
5 involving Hymenoptera. Consistent with the application of the IOBC scores (Table 21,
6 Figure 8), the Hemiptera appear to be substantially less sensitive relative to the Lepidoptera—
7 i.e., the points for Hemiptera in Figure 9 are right-shifted from points for Lepidoptera. The
8 tolerance of Hemiptera to spinosad is noted in the open literature (Baur et al. 2003; Eelen et al.
9 2006; Elzen and Elzen 1999; Martinou et al. 2014); furthermore, a matched direct spray assay of
10 a hymenopteran (*Encarsia formosa*, Aphelinidae) and hemipteran (*Orius insidiosus*,
11 Anthocoridae) notes the greater tolerance of the Hemiptera (Jones et al. 2005). While spinosad
12 is classified as highly toxic to *Orius insidiosus* (Hemiptera: Anthocoridae) in petri dish assays,
13 toxic effects are not documented in the more realistic exposures in field and greenhouse assays
14 (Studebaker and Kring 2003, Table 1).

15
16 The data on Coleoptera are based on only two studies, and the data on Diptera and Orthoptera are
17 based on only a single study each. These data are not sufficient to assess sensitivities relative to
18 Lepidoptera.

19 **4.1.2.4.6. Insect Resistance**

20 The spinosyns (i.e., both spinosad and spinetoram) are classified by the IRAC Resistance Action
21 Committee as Group 5: Nicotinic acetylcholine receptor (nAChR) allosteric modulators (IRAC
22 2016). This mode of action classification is unique to the spinosyns. A variety of related
23 nAChR competitive modulators (rather than allosteric) modulators—e.g., neonicotinoids and
24 sulfoximines—are classified as a mechanistically distinct group from the spinosyns in terms of
25 mechanisms for resistance.

26
27 Resistance often has an impact on efficacy. As noted in Section 4.1.2.4.5, however, efficacy is
28 not a focus of the current risk assessment. Nonetheless, the potential for resistance in
29 populations of the same or closely related species complicates the current risk assessment in that
30 resistance (or more generally variability in sensitivity among different populations) confounds
31 the assessment of systematic differences in sensitivity among different groups of terrestrial
32 invertebrates. For example, as discussed in Section 4.1.2.4.3 and summarized in Table 19,
33 differences in contact LD₅₀ values vary by a factor of about 12 [3.97 mg/kg bw ÷ 0.33 mg/kg bw
34 ≈ 12.03] for Diptera: Tephritidae and by a factor of about 27 [0.67 mg/kg bw ÷ 0.025 mg/kg bw
35 = 26.8] for *Apis mellifera*.

36
37 Resistance studies in four orders of target insects (i.e., Coleoptera, Diptera, Lepidoptera, and
38 Thysanoptera) are summarized in Table 24. Resistance is typically quantified as resistance
39 factors or ratios—i.e., the ratio of a dose associated with a defined response (e.g., LC₅₀) in
40 resistant populations to the dose associated with the same response in a sensitive population.
41 Note that some of the resistance factors given in Table 24 are less than one. In all cases, these
42 are examples of the investigators calculating the resistance factor as the ratio of the toxicity value
43 for a field population to the corresponding toxicity value for a laboratory population. Thus,
44 resistance factors of less than one simply indicate that the field population is more sensitive than
45 the laboratory reference population.

46

1 As noted in Table 22, resistance factors for spinosad range up to nearly 3 million (i.e.,
2 *Frankliniella occidentalis* in the study by Bielza et al. 2007). This and several other studies
3 summarized in Table 22 involve the artificial generation of resistance developed by subjecting
4 multiple generations of insect populations to lethal doses (i.e., LD₅₀ to LD₉₀) of spinosad and
5 breeding subsequent generations with the survivors of the bioassays. These types of exposures
6 are not likely to occur in the environment, and the very high resistance factors may be viewed as
7 physiological maximum potential resistance factors. The studies using artificial resistance
8 pressure are given in bold font in Table 22.

9
10 Other types of studies summarized in Table 22 involve simpler comparisons of field populations
11 (presumably subject to selection pressures in the normal use of spinosad) to laboratory
12 populations not subject to artificial selection pressure (e.g., Huang et al. 2004; Hsu et al. 2012a).
13 Most of the studies that focus on natural field populations of resistant insects note only moderate
14 resistance factors in the range of about 0.6 to 13 (Achaleke et al. 2009; Huang et al. 2004; Hsu et
15 al. 2012a; Scott 1998; Zhang et al. 2014). In some instances, the low reported resistance factors
16 may reflect simple variability in field populations rather than true resistance (i.e., Huang et al.
17 2004). Some field populations of Diptera, however, have much greater resistance factors—i.e.,
18 *Musca domestica* from the study by (Gao et al. 2007a) and some populations of *Drosophila*
19 *melanogaster* from the study by Rinkevich and Scott (2013). In addition, resistance factors of up
20 to nearly 2000 were observed in field populations of thrips (*Thrips palmi*) not subject to artificial
21 selection pressure (Bao et al. 2014).

22
23 The mechanisms of resistance are unclear. Some studies associate resistance at least partially
24 with an increased detoxification by cytochrome P450 isozymes (Bao et al. 2014; Markussen and
25 Kristensen 2012; Sayyed et al. 2008). Several other studies note no apparent relationship of
26 P450 activity with resistance and suggest that the primary mechanism of resistance involves
27 changes in the underlying receptor site (Bielza et al. 2007; Hsu et al. 2012b; Gao et al. 2007a;
28 Shi et al. 2011; Campos et al. 2014). Many studies indicate that resistance is a stable trait in the
29 absence of cross-breeding (Bielza et al. 2007) but appears to be a recessive trait if the resistant
30 populations crossbreed with non-resistant populations (Campos et al. 2014; Hou et al. 2014).
31 According to several studies, spinosad resistance may be a recessive trait due to increased energy
32 requirements, alterations in immune function, or delayed developmental effects associated with
33 the resistance to spinosad (Sayyed et al. 2008; Sagri et al. 2014).

34
35 Consistent with the presumably unique mechanism of resistance, at least four studies note that
36 resistance to spinosad is not associated with cross-resistance to other pesticides and resistance to
37 other pesticides is not associated with cross-resistance to spinosyns (Achaleke et al. 2009; Bielza
38 et al. 2007; Hsu and Feng 2006; Hussain et al. 2009).

39
40 Resistance to spinosad is noted also in aquatic assays of mosquito larvae (Khan et al. 2011a; Liu
41 et al. 2004a,b), as discussed further in Section 4.1.3.3.

42 **4.1.2.4.7. Earthworms**

43 The toxicity of spinosad to earthworms is not addressed in the open literature, including standard
44 compendia of earthworm toxicity studies (i.e., Edwards and Bohlen 1992; Potter et al. 1990,
45 1994; Wang et al. 2012).

1 The earthworm is the standard test species used by the EPA to assess the potential hazards to soil
2 invertebrates (U.S. EPA/OCSP 2012b). The most recent EPA ecological risk assessment
3 includes a brief summary of a 14-day soil bioassay in *Eisenia foetida* in which a concentration of
4 970 mg a.i./kg soil was not associated with signs of toxicity based on biomass (U.S.
5 EPA/OPP/EFED 2011a, p. 35, MRID 43414548). As summarized in Table 5, the toxicity value
6 of 970 mg a.i./kg soil is given on the Material Safety Data Sheets for spinosad as an indefinite
7 LC₅₀ (i.e., LC₅₀ > 970 mg a.i./kg soil).

8
9 A review of spinosad by the European Commission (2006, p. 31) notes an acute LD₅₀ for
10 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
11 LC₅₀ of >500 mg/kg soil for N-demethylated spinosyn D. In addition, the review by the
12 European Commission notes a reproductive NOEC of >2700 g a.i./ha (≈2.4 lb a.i./acre) for
13 NAF-85 and a reproductive NOEC for N-demethylated spinosyn D of >964 mg/kg soil. Details
14 of these unpublished studies are not given in European Commission (2006) review.

15
16 While HQs for earthworms are not typically derived in Forest Service risk assessments, the scant
17 data on earthworms are considered further in the dose-response assessment (Section 4.3.2.5.4)
18 and risk characterization (Section 4.4.2.4.4).

19 **4.1.2.5. Terrestrial Plants (Macrophytes)**

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

28
29 As specified in Appendix 4, the Tier 1 studies were conducted at an application rate of 0.5 lb
30 a.i./acre. In the EPA's more recent assessment for the registration review of spinosad, however,
31 the EPA notes that the available Tier 1 studies were not conducted at the maximum registered
32 application rate (≈0.8 lb a.i./acre). Thus, the EPA is requiring an *...acceptable tier I study is*
33 *needed that tests the effects of the maximum labeled application rate to terrestrial plants* (U.S.
34 EPA/OPP/EFED 2011a, p. 56). Note that the EPA is not requiring Tier 2 testing.

35
36 This data reservation is noted for the sake of transparency but does not impact the current Forest
37 Service risk assessment. As discussed in Section 2, the maximum seasonal application rate
38 proposed by the Forest Service is 0.45 lb a.i./acre—i.e., two applications of 0.225 lb a.i./acre.

39
40 As discussed in previous sections, spinosad has been applied to many species of plants for the
41 control of insect pests with no apparent adverse effects. In the absence of documented
42 phytotoxicity and given that the available Tier 1 studies are above the application rates proposed
43 by the Forest Service, toxicity to terrestrial vegetation is not identified as a potential hazard.

1 **4.1.2.6. Terrestrial Microorganisms**

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

8
9 The European Commission (2006, p. 31) provides a brief summary of unpublished studies on the
10 toxicity of spinosad to soil microorganisms, which indicates that spinosad, at a soil concentration
11 of 7.2 mg/kg soil, caused a transient decrease (-55%) in soil nitrification after 15 days but that
12 the effect was <25% at “*test termination*” (not otherwise specified). No substantial effects were
13 noted on carbon mineralization at 7.2 mg/kg and no effects on either nitrogen or carbon
14 mineralization were noted at 0.72 mg/kg soil. N-demethylated spinosyn D caused no effects on
15 nitrogen or carbon mineralization at concentrations of 0.3855 or 1.928 mg/kg soil. As discussed
16 further in the risk characterization (Section 4.4.2.6), the anticipated levels of spinosad in soil
17 following one or two applications at 0.225 lb a.i./acre are below the 0.72 mg/kg NOAEC noted
18 in European Commission (2006).

19 **4.1.3. Aquatic Organisms**

20 **4.1.3.1. Fish**

21 **4.1.3.1.1. Acute Toxicity**

22 Studies on the acute toxicity of spinosad and spinosad formulations in fish are summarized in
23 Appendix 5, Table A5-1. The U.S. EPA typically uses 96-hour LC₅₀ values in fish to assess the
24 potential for acute risks to fish. Based on the 96-hour LC₅₀ of 5.94 mg a.i./L in bluegill sunfish,
25 the EPA classifies spinosad as moderately toxic to bluegill sunfish (MRID 43414534). Based on
26 the 96-hour LC₅₀ of 30 mg a.i./L in rainbow trout, the EPA classifies spinosad as slightly toxic to
27 trout (MRID 43414534) (MRID 43444103) (U.S. EPA/OPP/EFED 2011a, p. 30). The lowest
28 reported 96-hour LC₅₀ is 4 mg a.i./L in carp. Carp is not a standard test species used by EPA.
29 The LC₅₀ in carp is taken from the review by the European Commission (2006). Details of this
30 study are not given on the European Commission review. As summarized in Table 5, the
31 Material Safety Data Sheets for the representative formulations of spinosad considered in the
32 current risk assessment specify LC₅₀ values in fish as 0.1 to 1 mg/L. These toxicity values are
33 not included in the EPA ecological risk assessments on spinosad.

34
35 Two indefinite acute LC₅₀ values are available on spinosad formulations—i.e., a 96-hour LC₅₀ of
36 >49 mg a.i./L for carp (European Commission 2006) and a 96-hour LC₅₀ of >500 mg/L for Coho
37 salmon from Deardorff and Start (2009). Based on the data on carp for both technical grade
38 spinosad and the NAF-85 formulation, it does not appear that other ingredients in the
39 formulation contribute to the toxicity of spinosad.

40
41 The effects of many pesticides and other chemicals include general signs of oxidative stress
42 typically characterized by an increase in free radical production and other reactive oxygen
43 species leading to increased lipid peroxidation, generalized tissue damage, cell death, and
44 depletion of endogenous antioxidants such as glutathione. General oxidative damage is a

1 common effect noted in mammals (Abdollahi et al. 2004; Agrawal and Sharma 2010) as well as
2 fish (Slaninovic et al. 2009; Stoliar and Lushchak 2012). As summarized in Appendix 5, Table
3 A3-1, Pine and Uner (2013, 2014) observed biochemical markers indicative of oxidative stress in
4 tilapia at spinosad concentrations of 25 mg a.i./L or greater using Laser, a 480 g a.i./L spinosad
5 formulation. The studies by Pine and Uner (2013, 2014) were conducted in Turkey. The Laser
6 formulation used in these studies is produced by the Dow Chemical Company
7 ([http://msdssearch.dow.com/PublishedLiteratureDAS/dh_092e/0901b8038092e97b.pdf?filepath](http://msdssearch.dow.com/PublishedLiteratureDAS/dh_092e/0901b8038092e97b.pdf?filepath=it/pdfs/noreg/011-04057.pdf&fromPage=GetDoc)
8 [=it/pdfs/noreg/011-04057.pdf&fromPage=GetDoc](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
9 United States (i.e., the formulation is not listed at the CDMS website (<https://www.cdms.net/>),
10 and U.S. labels for this formulation have not been identified. In any event, the concentration of
11 25 mg a.i./L—i.e., the lowest concentration assayed in the studies by Pine and Uner (2013,
12 2014—is more than 10 times higher than the NOAECs for fish on which the dose-response
13 assessment is based (Section 4.3.3.1.1).

14 **4.1.3.1.2. Longer-term Toxicity**

15 Studies on the longer-term toxicity of spinosad to fish are summarized in Appendix 5, Table
16 A5-2. Two of these studies are standard early life-stage studies submitted to the EPA and
17 summarized in the most recent EPA ecological risk assessments (U.S. EPA/OPP/EFED 2005,
18 2009a, 2011a)—i.e., an assay in trout (MRID 43414541) and an assay in sheepshead minnow
19 (MRID 44420601). A full DER is available for the assay in trout (Weinberg et al. 1993). In
20 these studies, trout were somewhat more sensitive (NOAEC = 0.498 mg a.i./L) than sheepshead
21 minnow (NOAEC = 1.15 mg a.i./L).

22
23 The review by Cleveland et al. (2002b) briefly summarizes a 21-day study in trout, reporting a
24 NOAEC of 1.2 mg/L. A full citation for this study is not given in the review, and this study
25 is not cited in EPA risk assessments. The 21-day study in trout is simply designated as a ...*21-*
26 *day flow-through*. Because the *21-day flow-through* study reports a higher NOAEC than the
27 early-life state study reviewed by EPA (MRID 43414541), the *21-day flow-through* study is cited
28 for the sake of completeness but is not otherwise used in the current Forest Service risk
29 assessment.

30
31 Two specialized studies in fish are published in the open literature (Anogwih et al. 2003; Elskus
32 2007). The study by Anogwih et al. (2002) is a micronucleus assay using mosquito fish
33 exposure to spinosad (NOS) at concentrations of up to 0.361 mg/L. Micronucleus assays are
34 sometimes used as mutagenicity screening tests. As discussed in Section 3.1.10, spinosad was
35 assayed adequately for both carcinogenicity and mutagenicity, and these endpoints are not a
36 concern—i.e., the assays for carcinogenicity and mutagenicity noted no positive activity. While
37 Anogwih et al. (2003) note some statistically significant differences in nuclear morphology
38 between control and treatment groups, the effects do not appear to be concentration dependent
39 (Figure 1 of paper).

40
41 The study by Elskus (2007) is an assay for effects on immune function in zebra fish embryos.
42 No responses suggestive of an effect on immune function were noted over the range of
43 concentrations assayed (i.e., 0.2 to 30 ppb or µg/L). As discussed in Section 3.1.7, the lack of an
44 immunotoxic response in fish is consistent with the determination in U.S. EPA/OPP/HED
45 (2011a) that concern for the immunotoxicity of spinosad is low.

1 **4.1.3.2. Amphibians (Aquatic Phase)**

2 As with terrestrial phase amphibians, there are no data on the toxicity of spinosad to aquatic
3 phase amphibians. The EPA ecological risk assessments on spinosad do not cite any registrant-
4 submitted studies on aquatic-phase amphibians (i.e., U.S. EPA/OPP/EFED 2005, 2009a, 2010a,
5 2011a). The general lack of toxicity data on aquatic-phase amphibians extends to the open
6 literature and the compendia of amphibian toxicity studies by Pauli et al. (2000). As noted in the
7 EPA's most recent risk assessment on spinosad (U.S. EPA/OPP/EFED 2011a, p. 46), the EPA
8 uses fish as a surrogate for aquatic-phase amphibians.

9 **4.1.3.3. Aquatic Invertebrates**

10 **4.1.3.3.1. Acute Toxicity**

11 Studies on the acute toxicity of spinosad to aquatic invertebrates are summarized in Appendix 5,
12 Table A5-1. Acute toxicity values expressed in units of water concentration consist primarily of
13 LC₅₀ values (concentrations estimated to cause 50% mortality) and EC₅₀ values (concentrations
14 estimated to cause a non-lethal response in 50% of the organisms assayed) for aquatic
15 invertebrates. An overview of the studies reporting acute LC₅₀ or EC₅₀ values is given in
16 Table 25. For aquatic invertebrates, the distinction between LC₅₀ and EC₅₀ values is often
17 unclear in publications, and the two terms may be used loosely and sometimes interchangeably.
18 For very small invertebrates, EC₅₀ values based on immobility can be readily determined while
19 LC₅₀ values (often based on lack of heart beat) may be difficult to determine. Cleveland et al.
20 (2002b) as well as some studies on individual spinosyns (discussed further below) report both
21 LC₅₀ and EC₅₀ values for *Daphnia magna*. As would be expected, the LC₅₀ values are
22 substantially higher than EC₅₀ values. When both LC₅₀ and EC₅₀ values are available, the
23 current discussion focusses on EC₅₀ values. Functionally, immobility in a natural environment is
24 equivalent to mortality. As discussed further in Section 4.3.3, the dose-response assessment for
25 aquatic invertebrates is concerned primarily with estimated no effect levels; however, LC₅₀ and
26 EC₅₀ values are generally preferable in estimating differences in sensitivity among species (e.g.,
27 Awkerman et al. 2008).

28 **4.1.3.3.1.1. Daphnids**

29 *Daphnia magna* is the most common freshwater invertebrate used in EPA risk assessments.
30 Based on the EC₅₀ of 14 mg a.i./L for spinosad (Milazzo et al. 1994, MRID 43574502), the EPA
31 classifies spinosad as *slightly toxic* to freshwater invertebrates (U.S. EPA/OPP/EFED 2011a,
32 p.31). As summarized in Table 5, some Material Safety Data Sheets give an LC₅₀ of 1.5 mg
33 a.i./L for spinosad in *Daphnia magna*. This toxicity value is not referenced in any of the EPA
34 risk assessments on spinosad.
35

36
37 As summarized in Appendix 4, Table A5-1, toxicity studies in *Daphnia magna* were conducted
38 on spinosyn A and spinosyn D—i.e., the components of spinosad—as well as several degradates
39 of spinosad. Of the two components of spinosad, spinosyn A (the major component) is less toxic
40 than spinosyn D (the minor component). Spinosyn A is classified as *Practically Nontoxic* based
41 on an indefinite EC₅₀ of >197 mg/L (MRID 46505307). Spinosyn D is classified as *Moderately*
42 *Toxic* based on a definitive EC₅₀ of 3.8 mg/L (MRID 46505307). Spinosyn B, a demethylated
43 degradate of spinosyn A, is also somewhat more toxic than spinosad and is also classified as
44 *Moderately Toxic* based on an EC₅₀ values of 6.5 mg/L (MRID 46505312) and 6.49 mg/L
45 (MRID 44597731).

1
2 While the data on *Daphnia magna* from registrant-submitted studies are relatively
3 straightforward, the open literature on spinosad in daphnids and other invertebrates is more
4 complex and (for some species) inconsistent. As summarized in Table 25, the lowest EC₅₀ for
5 spinosad is 0.0018 mg a.i./L based on a bioassay of Success[®] formulation in *Ceriodaphnia dubia*
6 (Deardorff and Stark 2009). This study was conducted at Washington State University and was
7 sponsored by the National Oceanic and Atmospheric Administration (NOAA). As summarized
8 in Table 3, Success[®] is one of the representative formulations of spinosad covered in the current
9 Forest Service risk assessment. Thus, at least for acute toxicity, the low EC₅₀ in *Ceriodaphnia*
10 *dubia* is relevant to the current risk assessment. As also summarized in Table 25 and detailed in
11 Appendix 5, Table A5-1, Deardorff and Stark (2009) also conducted assays with the Success[®]
12 formulation in *Daphnia magna* and *Daphnia pulex*. *Daphnia pulex* was the most tolerant of the
13 three species of daphnids with an EC₅₀ of 0.129 mg a.i./L. *Daphnia magna*, with an EC₅₀ of
14 0.0048 mg a.i./L was more sensitive than *Daphnia pulex* but less sensitive than *Ceriodaphnia*
15 *dubia*. Note that the EC₅₀ for *Daphnia magna* from this study is lower than the EC₅₀ of 14 mg/L
16 from the registrant-submitted study used by EPA (MRID 43574502) by a factor of nearly 3000
17 [14 mg/L ÷ 0.0048 mg a.i./L ≈ 2916.66]. This substantial variability suggests that components in
18 the Success[®] formulation other than spinosad contribute substantially to the toxicity of the
19 formulation to *Daphnia magna*. As indicated in Table 3, one known inert ingredient in Success
20 is propylene glycol (4% of the formulation). As summarized in HSDB (2015), propylene glycol
21 has a very low toxicity to daphnids with an EC₅₀ of >10,000 mg/L in *Daphnia magna* and 18,340
22 mg/L in a *Ceriodaphnia* species. Thus, it does not seem likely that propylene glycol could
23 account for the higher toxicity of the Success[®] formulation relative to technical grade spinosad.
24 Deardorff and Stark (2009) also examined the joint action of a surfactant (i.e., R-11) with the
25 Success[®] formulation and note that the surfactant may enhance the toxicity of the formulation to
26 *Ceriodaphnia dubia*. It is not known whether the Success[®] formulation contains a surfactant.

27 28 **4.1.3.3.1.2. Mosquitoes**

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

33
34 Most of the available mosquito studies were conducted with *Culex quinquefasciatus* and are
35 reasonably consistent with LC₅₀ values generally ranging from 0.01 to 0.031 mg/L—i.e., a range
36 that spans a factor of about 3. The LC₅₀ of 0.1 mg a.i./L for technical grade spinosad is
37 somewhat but not remarkably atypical. Jiang and Mulla (2009) matches studies on technical
38 grade spinosad and an 11.6% a.i. formulation. The formulation is modestly more toxic than
39 technical grade spinosad—i.e., an EC₅₀ of 0.019 mg/L for technical grade versus an EC₅₀ of
40 0.01 mg a.i./L for the formulation in 2nd instar larvae and an EC₅₀ of 0.026 mg/L for technical
41 grade versus an EC₅₀ of 0.013 mg a.i./L for the formulation in 4th instar larvae. Similarly,
42 Kovendan et al. (2012) note that earlier instar larvae are somewhat more sensitive than later
43 instar larvae to spinosad—see Appendix 5, Table A5-1 for details. As discussed in Section
44 4.1.3.3.1.3, this pattern was observed also in midge larvae (Kumar et al. 2011). This is a typical
45 pattern in aquatic toxicology with smaller organisms generally being more sensitive than larger

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

3
4 While the toxicity studies on *Culex quinquefasciatus* are reasonably consistent, the EC₅₀ values
5 for other species are less so. As summarized in Table 25, the reported EC₅₀ values range by a
6 factor of over 7000 for *Aedes aegypti* [51.7 mg/L ÷ 0.007 mg/L ≈ 7,386], a factor of about 16 for
7 *Aedes albopictus* [0.3 mg/L ÷ 0.019 mg/L ≈ 15.79], a factor of 12 for *Anopheles stephensi* [0.024
8 mg/L ÷ 0.002 mg/L = 12], and a factor of about 27 for *Culex pipiens* [0.087 mg/L ÷ 0.0032 mg/L
9 ≈ 27.18].

10
11 For *Aedes aegypti*, the very wide range in the toxicity values is due to the atypical EC₅₀ of 51.7
12 mg a.i./L reported by Kovendan et al. (2012). This study was conducted using eggs collected
13 from a field in India using a form of spinosad specified in the publication as material ... *obtained*
14 *from T-Stanes & Company Limited, Research and Development Centre, Coimbatore, Tamil*
15 *Nadu, India*. It is not clear if the spinosad was technical grade or a formulation. In addition, it is
16 not clear if the population of *Aedes aegypti* was subject previously to substantial exposures to
17 spinosad. As discussed further below, mosquitoes can develop resistance to spinosad but it does
18 not seem likely that the high EC₅₀ for *Aedes aegypti* would be due to resistance in a field
19 population. Disregarding the study by Kovendan et al. (2012), the other three LC₅₀ values for
20 this species are consistent with the data on *Culex quinquefasciatus* as well as the data on
21 *Daphnia magna* (4.1.3.3.1.1) indicating that the formulations are more toxic than technical grade
22 spinosad. This pattern is to be expected for insecticide formulations used to control mosquito
23 larvae, since it is reasonable to suppose that formulators would add other ingredients to the
24 formulation to enhance the control of mosquito larvae.

25
26 The variability in toxicity values for *Aedes albopictus* is based on only two studies: a 24-hour
27 EC₅₀ of 0.3 mg a.i./L using technical grade spinosad (Liu et al. 2004b) and a 48-hour EC₅₀ of
28 0.019 mg a.i./L using the Tracer® 24SC formulation (Khan et al. 2011). While this comparison
29 is consistent with the greater toxicity of formulations relative to technical grade spinosad, the
30 differences could also be due, at least partly, to the differences in exposure durations on which
31 the EC₅₀ values are based and differences in the sensitivity of the mosquito populations used in
32 the studies.

33
34 As summarized in Table 24 and discussed in Section 4.1.2.4.6, resistance to spinosad is well
35 documented in terrestrial insects with resistance factors of nearly 3 million in populations subject
36 to artificial selection pressure in the laboratory and resistance factors of somewhat over 7000 in
37 field populations. Resistance to spinosad in populations of mosquito larvae has also been
38 demonstrated, although the number of studies in mosquito larvae is fewer than the number of
39 studies in terrestrial insects. Under laboratory conditions with artificial selection pressure—i.e.,
40 45 generations of *Culex quinquefasciatus* subject to spinosad concentrations equivalent to
41 LC₇₀₋₉₀ values, resistance factors of somewhat over 1000 were noted (Su and Chen 2014b). In
42 field populations not subject to artificial selection pressure, resistance factors of 23 to 50 were
43 noted in populations of *Aedes albopictus* in Pakistan (Khan et al. 2011a), and resistance factors
44 of 0.7 to 3 were noted in populations of *Culex quinquefasciatus* in Alabama (Jones and Ottea
45 2013; Liu et al. 2004a,b). Additional details of these studies are given in Appendix 5, Table
46 A5-1.

1
2 Various factors can confound the assessment of species sensitivity differences in mosquito
3 populations, including prior exposures of the insects to spinosad and/or other spinosyns,
4 differences in the form of spinosad to which the mosquitos were exposed, and other experimental
5 details. The study by Romi et al. (2006) is exceptional in that it provides matched bioassays on
6 three species of mosquitoes. As summarized in Table 25, the order of sensitivities (most
7 sensitive to least) is: *Culex pipiens* ($EC_{50} = 0.0032$ mg a.i./L), *Aedes aegypti* ($EC_{50} = 0.007$ mg
8 a.i./L), and *Anopheles stephensi* ($EC_{50} = 0.024$ mg a.i./L). These bioassays were conducted with
9 the same formulation (Laser®, 4.8% EC) using 3rd instar larvae from laboratory populations
10 cultured for over 30 years.

11 12 **4.1.3.3.1.3. Other Aquatic Invertebrates**

13 In addition to the toxicity values for daphnids and mosquitoes, Table 25 summarizes acute
14 toxicity values for midge larvae (*Chironomus circumdatus*), the eastern oyster (*Crassostrea*
15 *virginica*), and grass shrimp (*Palaemonetes pugio*). Additional details for these studies are
16 given in Appendix 6, Table A6-1. Based on the study by Kumar et al. (2011), midge larvae
17 ($EC_{50} = 0.009$ mg/L) are nearly as sensitive as some daphnids and sensitive species of
18 mosquitoes. The eastern oyster is much less sensitive with an EC_{50} of 0.3 mg/L (MRID
19 43571203). As indicated in the review by Cleveland et al. (2002b), the endpoint for the EC_{50} in
20 oysters is new shell growth rather than immobility or mortality. The grass shrimp is highly
21 tolerant with an LC_{50} of >9.67 mg/L (Cleveland et al. 2002b). Grass shrimp are much larger than
22 daphnids, mosquitoes, and midge larvae, and the tolerance of the grass shrimp to spinosad is
23 consistent with the general pattern of small aquatic invertebrates being more sensitive than larger
24 aquatic invertebrates (Section 4.1.3.3.1.2). This study is cited in the U.S. EPA/OPP/EFED risk
25 assessment on spinosad (2005, MRID 434145-39) and used to classify spinosad as *moderately*
26 *toxic*.

27
28 In addition to the standard bioassays, other non-standard studies on the toxicity of spinosad to
29 aquatic invertebrates are summarized at the end of Appendix 6, Table A6-1. Jones and Ottea
30 (2013) examined mortality in three groups of nontarget aquatic invertebrates: damselflies
31 (*Ischnura* sp., Odonata: Coenagrionidae), dragonflies, (*Pachydiplax longipennis*, Odonata:
32 Libellulidae); and mayflies (*Caenis* sp., Ephemeroptera: Caenidae). The bioassays were
33 conducted at two concentrations: 0.031 mg a.i./L (the LC_{50} for 3rd instar larvae of *Culex*
34 *quinquefasciatus* as assayed by these investigators) and 1.6 mg a.i./L (the maximum application
35 rate calculated by the investigators for a spinosad formulation used to control mosquito larvae).
36 In these assays, mayflies were the most sensitive group with mortality in excess of 50% at both
37 concentrations (Figure 2 of paper)—i.e., the mayflies appeared to be more sensitive than the
38 mosquito species. Of the two Odonata species, damselflies were more sensitive than dragon
39 flies; however, both species of Odonata appeared to be less sensitive than the mosquito species.

40
41 Infante-Rodriguez et al. (2011) examined the potential efficacy of spinosad for the control of
42 black fly larvae (*Simulium* sp., Diptera: Simuliidae) in a series of short-term (10-minute pulse
43 exposures) to black fly larvae as well as various groups of nontarget aquatic insect larvae. The
44 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,
45 a species of stonefly (*Anacronaura* sp., Plecoptera: Perlidae) was the only nontarget for which a
46 significant increase in mortality was observed. A significant increase in mortality relative to

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

4 **4.1.3.3.2. Longer-term Toxicity**

5 **4.1.3.3.2.1. Spinosad**

6 Information on the chronic toxicity of spinosad to aquatic invertebrates is summarized in
7 Appendix 6, Table A6-2. An overview of these studies is given in Table 26. Note that units of
8 $\mu\text{g/L}$ rather than mg/L (used for acute studies) are used in Table 26 and in the following
9 discussion because of the much lower toxicity values in chronic relative to acute exposures.

10
11 As discussed in Section 4.1.3.3.1 and summarized in Table 25, the available acute toxicity
12 studies suggest that spinosad formulations may be more toxic than technical grade spinosad to
13 daphnids. In addition, the study by Deardorff and Stark (2009) indicates that *Ceriodaphnia*
14 *dubia* is substantially more sensitive than *Daphnia magna*. Neither of these patterns is apparent
15 in the available chronic studies on daphnids. As summarized in Table 26, Deardorff and Stark
16 (2011) conducted a reproduction study in *Ceriodaphnia dubia* using the same Success[®]
17 formulation used in the acute toxicity studies. The chronic NOAEC of 0.5 $\mu\text{g a.i./L}$ in
18 *Ceriodaphnia dubia* is not substantially different from the chronic NOAEC of 0.62 mg a.i./L in
19 *Daphnia magna* reported in U.S. EPA/OPP/EFED (2011a, MRID 43848801).

20
21 The European Commission (2006) reports somewhat higher NOAEC values in *Daphnia magna*
22 based on a flow-through assay (NOAEC = 1.2 $\mu\text{g/L}$) and a static renewal assay (NOAEC = 8 μg
23 a.i./L). The static renewal 21-day NOAEC of 8 $\mu\text{g/L}$ from the European Commission (2006) is
24 not consistent with the static renewal 14-day LOAEC of 8 $\mu\text{g/L}$ in *Daphnia magna* from the
25 study by Duchet et al. (2010b). As summarized in Appendix 6, Table A6-2, the review by
26 Cleveland et al. (2002b, Table 3 of paper) reports an NOEC of 6.88 $\mu\text{g a.i./L}$ for a ...21-day flow
27 through 5-day pulsed... exposure in *Daphnia magna*. Details of this study are not discussed in
28 the review by Cleveland et al. (2002b), and this study is not summarized in the EPA ecological
29 risk assessments.

30
31 In a somewhat unusual static renewal study, Stark and Vargas (2003) conducted a long-term
32 population study in *Daphnia pulex* at concentrations ranging from 2 to 11 $\mu\text{g a.i./L}$. At
33 concentrations of 10 and 11 $\mu\text{g a.i./L}$, all organisms died by about Day 10. At lower
34 concentrations, populations survived for up to about 70 days (see Figure 1 of paper). A decrease
35 in net reproductive rate (i.e., the number of offspring per generation) was observed at all
36 concentrations (Figure 3 of paper). The LOAECs in *Daphnia pulex* reported by Stark and
37 Vargas (2003) is supported by a LOAEC of 8 $\mu\text{g a.i./L}$ in *Daphnia pulex* (Duchet et al. 2010b).
38 Due to the lack of NOAELs from these studies, the sensitivity of *Daphnia pulex* relative to
39 *Daphnia magna* and *Ceriodaphnia dubia* cannot be assessed. In a subsequent publication, Stark
40 (2005) exposed a population (n=300) of *Daphnia pulex* to spinosad (source not specified) at a
41 concentration of 129 $\mu\text{g a.i./L}$. As summarized in Table 25, 129 $\mu\text{g a.i./L}$ is the 48 hour-LC₅₀ for
42 *Daphnia pulex* from the study by Deardorff and Stark (2009). As might be expected, none of the
43 *Daphnia pulex* survived the 10 day exposure to 129 $\mu\text{g a.i./L}$ (Stark S2005).

44
45 Table 25, also summarizes three chronic NOAECs for midge larvae. The lowest NOAEC is
46 0.622 $\mu\text{g a.i./L}$ reported in U.S. EPA/OPP/EFED (2011a, MRID 44828402). This NOAEC is

1 virtually identical to the NOAEC of 0.62 µg a.i. for *Daphnia magna* also reported in U.S.
2 EPA/OPP/EFED (2011a, MRID 43848801). Notably, the endpoint for chronic studies in midge
3 larvae involves adult emergence rather than reproduction. Both the European Commission
4 (2006) and Cleveland et al. (2002a) review report a chronic NOAEC in midge larvae of 1.6 µg
5 a.i./L. It seems likely that these identical NOAECs reflect a single study; however, that cannot
6 be determined clearly from the references cited in the European Commission (2006) and
7 Cleveland et al. (2002a).

8
9 While the NOAECs for *Daphnia magna* and midge larvae are similar, mysid shrimp (*Mysidopsis*
10 *bahia*) are much more tolerant, based on the reported chronic NOAEC of 84.2 µg a.i./L (U.S.
11 EPA/OPP/EFED 2011a, MRID 47702901).

12 13 **4.1.3.3.2.2. Components and Metabolites**

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

16
17 Based on NOAEC of 0.62 µg a.i./L in *Daphnia magna* (MRID 43848801), none of the
18 metabolites is more toxic than spinosad. The β-13,14-dihydropseudo-aglycone degradation
19 products of both spinosyn A (NOAEC = 4850 µg/L, MRID 46505303) and spinosyn D (NOAEC
20 = 1590 µg/L, MRID 46505305) are much more toxic than technical grade spinosad. Unlike the
21 case with acute toxicity in *Daphnia magna*, Spinosyn B, a demethylated degradate of spinosyn
22 A, as well as a demethylated degradate of spinosyn D are modestly less toxic than spinosad—
23 i.e., an NOAEC for spinosyn B of 0.95 µg/L and a NOAEC of 1 µg/L for N-demethylated
24 spinosyn D (European Commission 2006).

25
26 As with *Daphnia magna*, none of the data on the degradates of spinosad are shown to be more
27 toxic to midge larvae than spinosad itself based on NOAEC of 0.622 µg a.i./L in midge larvae
28 (MRID 44828402). On the other hand, there are two equivocal studies that report indefinite
29 NOAECs (i.e., studies in which LOAECs were not defined). These studies include an assay of
30 N-demethylated spinosyn D which yielded an indefinite NOAEC of 0.14 µg a.i./L (MRID
31 46505315) and an assay of N-demethylated spinosyn A which yielded an indefinite NOAEC of
32 0.41 µg a.i./L (MRID 46505315). All other studies on midge larvae using degradates of
33 spinosad yielded NOAECs higher than spinosad itself.

34
35 Also, as with acute toxicity in *Daphnia magna* (Section 4.1.3.3.1), one chronic study in midge
36 larvae indicates that spinosyn A, the major component in spinosad, is much less toxic than
37 technical grade spinosad—i.e., the chronic indefinite NOAEC in midge larvae of 73.4 µg a.i./L
38 (MRID 46505314).

39 **4.1.3.3.3. Microcosm/Mesocosm Studies**

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

1 6, Table A6-3, involve outdoor and relatively complex systems that could be referred as
2 mesocosms rather than outdoor microcosms (e.g., Suter and Bartell 1993).

3
4 None of the mesocosm studies notes effects that are inconsistent with the more controlled
5 chronic studies discussed in Section 4.1.3.3.2. In the 21-day mesocosm study on *Daphnia pulex*,
6 Duchet et al. (2008) note that adverse effects (i.e., decreased body length) occurred at
7 concentrations as low as 2 µg/L and view this as ...*inconsistent with the laboratory data*
8 *published by Stark (2005), who estimated the acute LC₅₀ at 129 µg/L* (Duchet et al. 2008, p. 76).
9 As summarized in Table 26, the study by Stark (2005) involves a 10-day exposure of *Daphnia*
10 *pulex* to 129 µg a.i./L, a concentration equivalent to the 48-hour LC₅₀ from the study by
11 Deardorff and Stark (2009). Nonetheless, the adverse effects on *Daphnia pulex* in the mesocosm
12 study by Duchet et al. (2008) at concentrations as low as 2 µg a.i./L are consistent with the
13 reproductive LOAEC of 2 µg a.i./L from the chronic study in *Daphnia pulex* (i.e., Stark and
14 Vargas 2003 as summarized in Table 26).

15
16 In a subsequent mesocosm study in *Daphnia magna*, Duchet et al. (2010a) observed decreases in
17 daphnid abundance at concentrations as low as 2 µg a.i./L over a 21-day period of exposure. As
18 summarized in Table 26, this observation is consistent with the LOAEC of 1.2 µg a.i./L and a
19 corresponding NOAEC of 0.62 µg a.i./L in *Daphnia magna* from MRID 43848801 as
20 summarized in U.S. EPA/OPP/ EFED (2011a) and Cleveland et al. (2002b). More recently,
21 Duchet et al. (2015) conducted a similar mesocosm study on two species of midge larvae
22 [Diptera: Chironomidae] and observed adverse effects (decreased emergence) at concentrations
23 of 8 µg a.i./L in *Polypedilum nubifer* and at 17 µg a.i./L in *Tanytarsus curticornis*. These species
24 were not assayed in standard chronic laboratory studies; nonetheless, the LOAECs from the
25 mesocosm study are substantially above the LOAEC of 1.328 µg a.i./L from the standard chronic
26 bioassay in *Chironomus riparius* (MRID 44828402 as summarized in Table 26 and detailed in
27 Appendix 6, Table A6-2).

28
29 Jiang and Mulla (2009) is a relatively standard efficacy study in mosquitoes, *Culex*
30 *quinquefasciatus*. The satisfactory control of mosquito larvae at concentrations of 50 µg a.i./L
31 and higher is consistent with the 48 hour-LC₅₀ of about 0.01 mg a.i./L in this species (Table 25).
32 The study by Lawler and Dritz (2013) expresses exposures in terms of application rates in lb/acre
33 rather than concentrations of spinosad in water. This study is interesting, however, in noting that
34 nontarget Ephemeroptera (i.e., *Callibaetis californicus* nymphs) are more sensitive than larvae of
35 the target mosquito species, *Culex tarsalis* [Diptera: Culicidae] and chironomid midge larvae.
36 The lesser sensitivity of Ephemeroptera relative to Diptera is noted in short-term studies by
37 Infante-Rodriguez et al. (2011); however, the opposite pattern is noted in acute toxicity studies
38 conducted by Jones and Ottea (2013) (Section 4.1.3.3.1.3).

39 **4.1.3.4. Aquatic Plants**

40 **4.1.3.4.1. Algae**

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

1 The most sensitive species is the freshwater diatom, *Navicula pelliculosa*, with EC₅₀ values for
2 technical grade spinosad ranging from 0.09 mg a.i./L (MRID 43414543) to 0.135 mg a.i./L
3 (Cleveland et al. 2002b). Notably, both of these studies report a NOAEC of 0.05 mg a.i./L; thus,
4 it seems reasonable to conclude that these are summaries of the same study (conducted by or for
5 Dow AgroSciences and then submitted to EPA) and that the relatively minor difference in the
6 EC₅₀ values reflect a reanalysis of the dose-response data by EPA. Based on the 5-day EC₅₀
7 values reported in the European Commission (2006) review for technical grade spinosad (EC₅₀ =
8 0.079 mg a.i./L) and the NAF-85 formulation (EC₅₀ = 0.35 mg a.i./L), the other ingredients in the
9 formulation do not appear to contribute to and may, in fact, reduce its toxicity to this species of
10 algae. While the European Commission (2006) does not describe the formulation other than
11 using the NAF-85 designation, this designation is identified in EPA/OPP/HED (1997b) as the
12 Tracer[®] formulation (44.2% a.i.).
13

14 As also summarized in Appendix 7, Table A7-1, *Navicula pelliculosa* has been used in bioassays
15 of several degradates of spinosad. Based on definitive EC₅₀ values, most of the degradates
16 appear to be much less toxic than spinosad with EC₅₀ values ranging from 0.16 mg a.i./L (N-
17 demethyl-A) to 38.8 mg a.i./L (the β-13,14-dihydropseudo-aglycone degradate of spinosyn A).
18 The only exception involves the toxicity of spinosyn B, demethylated degradate of spinosyn A.
19 Based on the EC₅₀ values given in the European Commission (2006) review, the spinosyn A
20 (EC₅₀ = 0.077 mg a.i./L) is about equitoxic to technical grade spinosad (0.079 mg a.i./L). This is
21 similar to the pattern in *Daphnia magna* in which spinosyn B was somewhat more toxic than
22 technical grade spinosad (Section 4.1.3.3.1.1).
23

24 Other species of algae are much less sensitive to spinosad with EC₅₀ values ranging from 0.227
25 mg a.i./L (*Skeletonema costatum*) to >105.5 mg a.i./L (*Selenastrum capricornutum*) with both of
26 these EC₅₀ values reported in Cleveland et al. (2002b). The minimal toxicity of spinosad to
27 *Selenastrum capricornutum* is supported by an indefinite EC₅₀ of >48 mg a.i. for the NAF-85
28 formulation (European Commission 2006).

29 **4.1.3.4.2. Aquatic Macrophytes**

30 The only information on the toxicity of spinosad to aquatic macrophytes is a standard 7-day
31 study in duckweed, *Lemna gibba* (MRID 43414546), that yielded an EC₅₀ for growth of 10.6 mg
32 a.i./L with a corresponding NOAEC of 1.86 mg a.i./L. This study is briefly summarized in U.S.
33 EPA/OPP/EFED (2011a) and Cleveland et al. (2002b). The open literature does not appear to
34 include other information on the toxicity of spinosad to aquatic macrophytes
35

1 **4.2. EXPOSURE ASSESSMENT**

2 **4.2.1. Overview**

3 A standard set of exposure assessments for terrestrial and aquatic organisms is provided in the
4 EXCEL workbooks for spinosad. Attachment 1 details the exposure assessments for foliar
5 applications at the anticipated application rate of 0.225 lb a.i./acre. Attachment 2 covers two
6 applications at the rate of 0.225 lb a.i./acre with a 6-day application interval. As with the
7 exposure assessment for human health (Section 3.2), all exposure assessments involving
8 applications of spinosad are expressed in units of active ingredient (a.i.).
9

10 As in the human health risk assessment, three general types of exposure scenarios are
11 considered: accidental, acute non-accidental, and longer-term. Exposure assessments are
12 detailed in Worksheet G01a for mammals and in Worksheet G01b for birds. For both mammals
13 and birds, the highest exposure scenarios are associated with the consumption of contaminated
14 vegetation. This is a common pattern for applications of any pesticide to vegetation. The highest
15 exposures are associated with the consumption of contaminated short grass by a small mammal
16 or bird.
17

18 Exposure scenarios for honeybees and phytophagous insects are also considered quantitatively.
19 Forest Service risk assessments of insecticides typically assess risks to honeybees based on a
20 direct spray scenario and pathways for direct spray and spray drift are considered. For
21 phytophagous insects and foraging honeybees, exposures are estimated, although the information
22 used to estimate exposures is based on different data sets for the two groups of terrestrial
23 invertebrates.
24

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

27 **4.2.2. Mammals and Birds**

28 All of the exposure scenarios that are more or less standard in Forest Service risk assessments for
29 broadcast applications are not relevant to the foliar and bark application methods considered in
30 the current risk assessment of spinosad.
31

32 Table 27 provides an overview of the mammalian and avian receptors considered in the current
33 risk assessment. These data are discussed in the following subsections. Because of the
34 relationship of body weight to surface area as well as to the consumption of food and water, the
35 dose for smaller animals is generally higher, in terms of mg/kg body weight, than the dose for
36 larger animals. Consequently, the exposure assessment for mammals considers five nontarget
37 mammals of varying sizes: small (20 g) and medium (400 g) sized omnivores, a 5 kg canid, a 70
38 kg herbivore, and a 70 kg carnivore. Four standard avian receptors are considered: a 10 g
39 passerine, a 640 g predatory bird, a 2.4 kg piscivorous bird, and a 4 kg herbivorous bird.
40 Because of presumed differences in diet, (i.e., the consumption of food items), all of the
41 mammalian and avian receptors are not considered in all of the exposure scenarios (e.g., the
42 640 g predatory bird is not used in the exposure assessments for contaminated vegetation).

1 **4.2.2.1. Direct Spray**

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

12
13 Exposure assessments for the direct spray of a large mammal are not developed. As discussed
14 further in Section 4.4.2.1, the direct spray scenarios lead to HQs far below the level of concern,
15 and an elaboration for body size would have no impact on the risk assessment.

16 **4.2.2.2. Dermal Contact with Contaminated Vegetation**

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

27
28 For spinosad, the failure to quantify exposures associated with dermal contact adds relatively
29 little uncertainty to the risk assessment, since the consumption of contaminated vegetation is the
30 greatest source of exposure, as discussed below (Section 4.2.2.3).

31 **4.2.2.3. Ingestion of Contaminated Vegetation or Prey**

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

37
38 The acute and chronic exposure scenarios are based on the assumption that 100% of the diet is
39 contaminated, which may not be realistic for some acute exposures and seems an unlikely event
40 in chronic exposures to birds or larger mammals which may move in and out of the treated areas
41 over a prolonged period of time. While estimates of the proportion of the diet contaminated
42 could be incorporated into the exposure assessment, the estimates would be an essentially
43 arbitrary set of adjustments. The proportion of the contaminated diet is linearly related to the
44 resulting HQs, and its impact is discussed further in the risk characterization (Section 4.4.2).

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

11
12 The estimates of field metabolic rates are used to calculate food consumption based on the
13 caloric value (kcal/day dry weight) of the food items considered in this risk assessment and
14 estimates of the water content of the various foods. Estimates of caloric content are summarized
15 in Table 28. Most of the specific values in Table 28 are taken from Nagy (1987) and U.S.
16 EPA/ORD (1993).

17
18 Along with the exposure scenarios for the consumption of contaminated vegetation, similar sets
19 of exposure scenarios are provided for the consumption of small mammals by either a predatory
20 mammal (Worksheet F10a) or a predatory bird (Worksheet F10b) and the consumption of
21 contaminated insects by a small mammal, a larger (400 g) mammal, and a small bird
22 (Worksheets F09a-c).

23 ***4.2.2.4. Ingestion of Contaminated Water***

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

29
30 Body weight and water consumption rates are the major differences in the exposure estimates for
31 birds and mammals, relative to humans. Like food consumption rates, water consumption rates,
32 which are well characterized in terrestrial vertebrates, are based on allometric relationships in
33 mammals and birds, as summarized in Table 27.

34
35 Like food consumption, water consumption in birds and mammals varies substantially with diet,
36 season, and many other factors. Quantitative estimates regarding the variability of water
37 consumption by birds and mammals are not well documented in the available literature and are
38 not considered in the exposure assessments. As discussed further in Section 4.4.2.1 (risk
39 characterization for mammals) and Section 4.4.2.2 (risk characterization for birds), exposures
40 associated with the consumption of contaminated surface water are far below the level of
41 concern (HQ=1). Consequently, extreme variations in the estimated consumption of
42 contaminated water by mammals and birds would have no impact on the risk characterization for
43 mammals and birds.

1 **4.2.2.5. Consumption of Contaminated Fish**

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

12
13 Spinosad exposure levels associated with the consumption of contaminated fish depend on the
14 spinosad concentration in water and the bioconcentration factor for spinosad in fish. The
15 concentrations of spinosad in water are identical to those discussed in Section 4.2.2.4. The
16 bioconcentration factor for whole fish is taken as 84 for acute exposures and a BCF of 115 for
17 longer-term exposures. As summarized in Table 2, these BCF values are within the range of
18 BCFs summarized in U.S. EPA/OPP/EFED (2009a, p. 8). Given the relationship between
19 exposure time and bioconcentration, the lower bound of 84 is used for acute exposures and the
20 upper bound of 115 is used for longer-term exposures.

21 **4.2.3. Terrestrial Invertebrates**

22 **4.2.3.1. Direct Spray and Drift**

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

27
28 Honeybees are used as a surrogate for other terrestrial insects, and honeybee exposure levels
29 associated with broadcast applications are modeled as a simple physical process based on the
30 application rate and planar surface area of the bee. The planar surface area of the honeybee (1.42
31 cm²) is based on the algorithms suggested by Humphrey and Dykes (2008) for a bee with a body
32 length of 1.44 cm.

33
34 The amount of a pesticide deposited on a bee during or shortly after application depends on how
35 close the bee is to the application site as well as foliar interception of the spray prior to
36 deposition on the bee. The estimated proportions of the nominal application rate at various
37 distances downwind given in G09 are based on Tier 1 estimates from AgDRIFT (Teske et al.
38 2002) for distances of 0 (direct spray) to 900 feet downwind of the treated site. Further details of
39 the use of AgDRIFT are discussed in SERA (2014a, 4.2.4.2) and SERA (2011b, Section 3.3.2).

40
41 In addition to drift, foliar interception of a pesticide may occur. The impact of foliar interception
42 varies according to the nature of the canopy above the bee. For example, in studies investigating
43 the deposition rate of diflubenzuron in various forest canopies, Wimmer et al. (1993) report that
44 deposition in the lower canopy, relative to the upper canopy, generally ranged from about 10%

1 (90% foliar interception in the upper canopy) to 90% (10% foliar inception by the upper canopy).
2 In Worksheet G09, foliar interception rates of 0% (no interception), 50%, and 90% are used.

3
4 During broadcast applications of a pesticide, it is likely that terrestrial invertebrates other than
5 bees will be subject to direct spray. As discussed in Section 4.1.2.4.1, summarized in Table 19,
6 and detailed further in Section 4.3.2.3 (dose-response assessment for terrestrial invertebrates),
7 toxicity data on other terrestrial invertebrates suggest that honeybees are the most sensitive
8 species of terrestrial invertebrates for which contact toxicity data are available.

9 **4.2.3.2. Ingestion of Contaminated Vegetation or Prey**

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

15
16 An estimate of food consumption is necessary to calculate a dose level for a foraging
17 herbivorous insect. Insect food consumption varies greatly, depending on the caloric
18 requirements in a given life stage or activity of the insect and the caloric value of the food to be
19 consumed. The derivation of consumption values for specific species, life stages, activities, and
20 food items is beyond the scope of the current analysis. Nevertheless, general food consumption
21 values, based on estimated food consumption per unit body weight, are readily available.

22
23 Reichle et al. (1973) studied the food consumption patterns of insect herbivores in a forest
24 canopy and estimated that insect herbivores may consume vegetation at a rate of about 0.6 of
25 their body weight per day (Reichle et al. 1973, pp. 1082 to 1083). Higher values (i.e., 1.28-2.22
26 in terms of fresh weight) are provided by Waldbauer (1968) for the consumption of various types
27 of vegetation by the tobacco hornworm (Waldbauer 1968, Table II, p. 247). The current risk
28 assessment uses food consumption factors of 1.3 (0.6 to 2.2) kg food /kg bw. The lower bound
29 of 0.6 is taken from Reichle et al. (1973), and the central estimate and upper bound are taken
30 from the range of values provided by Waldbauer (1968).

31
32 A summary of the estimated exposures in terrestrial herbivorous insects is given in Worksheet
33 G08a, and details of the calculations for these scenarios are provided in Worksheets G07a, G07b,
34 G07c, and G07d of the EXCEL workbooks that accompany this risk assessment (Attachments 1
35 and 2). These levels pertain to the four food items included in the standard residue rates
36 provided by Fletcher et al. (1994), as summarized in Table 15.

37 **4.2.3.3. Nectar Exposures Involving Honeybees**

38 **4.2.3.3.1 General Method**

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

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

7
 8 The basic algorithm for estimating the daily dose (D) to the foraging bee, based on the nutritional
 9 requirements of the bee is:

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

10 where:

11 C = Concentration of pesticide in nectar in units of mg/L

12 Am = Amount of nectar in liters consumed by a foraging bee per day based
 13 on the nutritional requirements of the bee.

14 BW = Body weight of the bee in kilograms.

15
 16
 17
 18 The amount of nectar a bee needs to consume is calculated from the nutritional requirements of
 19 the bee. Nutritional requirements for bees are generally expressed in the literature as the amount
 20 of sugar per unit time. Rortais et al. (2005) express the sugar requirement of bee during flight as
 21 8 - 12 mg/hour, which is reasonably close to the value of 11.5 mg/hour cited by Winston (1987).
 22 The current risk assessment uses a sugar requirement for flight of 10 (8 - 12) mg/hour.

23
 24 The number of hours/day that a bee might spend foraging is likely to be highly variable. Rortais
 25 et al. (2005) use a range from 4 to 10.7 hours/day. This range is used in the current exposure
 26 assessment on spinosad with a central estimate of 6.5 hours/day, the approximate geometric
 27 mean of the lower and upper bounds from Rortais et al. (2005).

28
 29 Thus, the amount(s) of sugar ($Am_{SugarFl}$) required by a bee to support flight activities during
 30 foraging is calculated as the product of the sugar requirements per hour during flight and the
 31 number of hours/day that the bee spends in flight:

$$Am_{Sugar\ FL} = Rate_{mg/h} \times Fight_{h/day} \quad (8)$$

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

32
 33
 34 Using the above equation, the amount(s) of sugar required per day to support flight activities is
 35 calculated as 65.5 (32 - 128.4) mg/day.

36
 37
 38 Rortais et al. (2005) base their exposure assessment only on sugar requirements during flight. In
 39 the current Forest Service risk assessment of spinosad, the estimated nutritional requirement also
 40 includes time at rest, using the value of 0.7 mg/hour from Winston (1987, p. 61). From the same
 41 equation used above, the sugar requirement(s) for hours other than those engaged in flight is
 42 calculated as:

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

1
2 which is equivalent to 12.25 (14 to 9.31) mg/day.

3
4 Thus, the total sugar requirement(s) per day for a foraging honeybee is calculated as:

5
6

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

7

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

11
12 The sugar content of nectar also varies among plants and locations. Rortais et al. (2005) uses a
13 value of 0.4—i.e., nectar consists of 40% w/w nutritional sugars. This single value is also used
14 in the current risk assessment. So, when the sugar requirement(s) is divided by 0.4 (mg
15 sugar/mg nectar), the estimated amount of nectar required per day is about 193 (115 - 344)
16 mg/day. In the worksheets for this exposure scenario (i.e., G10 in the attachments), these values
17 are converted to units of kg nectar per day by dividing mg/day by 1,000,000 mg/kg.

18
19 The exposure assessments in the EXCEL workbooks are based on honey and not nectar
20 consumption. This approach is inconsequential since the basis of the exposure assessment is the
21 energy requirement of the bee. As discussed by Rortais et al. (2005, p. 73, column 2),

22
23 *As we do not know the bees' differential consumption of nectar and honey,*
24 *we related their sugar consumption depending on whether they consume*
25 *nectar or honey. With the example of sunflower, when a honeybee requires*
26 *1 mg of sugar, it will have to consume either 2.5 mg of fresh sunflower*
27 *nectar or 1.25 mg of sunflower honey.*

28 – Rortais et al. 2005, p. 73

29
30 In other words, the amount of spinosad consumed by the bee would be the same whether the
31 exposure is based on nectar consumption or honey consumption.

32
33 Another uncertainty in the amount of contaminated nectar that a foraging honeybee might
34 consume involves the proportion of the plants that are contaminated in the area in which the
35 honeybee forages. For broadcast applications, this factor is inconsequential as it seems
36 reasonable to assume that 100% of the plants would be contaminated. More focused application
37 methods, such as directed foliar, could and probably would generally result in a highly uneven
38 distribution of spinosad over the general area in which the applications occur. Nonetheless, the
39 assumption used in the current risk assessment is that backpack applications would be done at
40 the nominal application rate of 0.225 lb a.i./acre which could be viewed as a functional average
41 over the treated area.

42 **4.2.3.3.2. Concentrations of Spinosad in Nectar**

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

1 480SC formulation at an application rate of 40 g a.i./ha (≈ 0.0357 lb a.i./acre), Bailey et al. (2005)
2 detected spinosad in sweet corn pollen at a concentration of 0.32 mg a.i./kg pollen (Bailey et al.
3 2005, p. 630, Table IV). In the absence of additional relevant information, a contamination rate
4 for pollen is calculated as about 8.96 mg a.i./kg pollen per lb a.i./acre [$0.32 \text{ mg a.i./kg pollen} \div$
5 $0.0357 \text{ lb a.i./acre} \approx 8.9636 \text{ mg a.i./kg pollen per lb a.i./acre}$].

6
7 The contamination rate for pollen is used to estimate a contamination rate for nectar using the
8 study by Dively and Kamel (2012). These investigators monitored concentrations of three
9 insecticides (i.e., imidacloprid, dinotefuran, and thiamethoxam) in both pollen and nectar of
10 flowering pumpkin plants (*Cucurbita pepo*). As summarized in Table 29, the ratios of nectar to
11 pollen covered a relatively narrow range (i.e., 0.08 to 0.16) with a mean and 95% confidence
12 interval of 0.12 (0.099 – 0.15). This mean and confidence interval on the ratios of nectar-to-
13 pollen are multiplied by the 8.96 mg a.i./kg pollen per lb a.i./acre to estimate a contamination
14 rate for nectar of 1.08 (0.89 – 1.34) mg a.i./kg nectar per lb a.i./acre.

15
16 The lack of field monitoring data on the concentrations of spinosad in the nectar of wild flowers
17 that might be foraged by bees following Forest Service applications of spinosad is an obvious
18 and substantial limitation, as discussed further in the risk characterization for pollinators (Section
19 4.4.2.4.3).

20 **4.2.3.3.3. Exposure Estimates**

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

31 **4.2.3.4. Concentrations in Soil**

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

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

3 **4.2.3.5. Contact with Contaminated Surfaces**

4 As in the human health risk assessment (Section 3.2.3.3) and vertebrate wildlife (Section
5 4.2.2.2), the contact of terrestrial invertebrates with contaminated vegetation is a potential
6 exposure route of concern. Insects are likely to come into contact with spinosad on contaminated
7 surfaces after directed or broadcast applications; however, data and methods to quantify this type
8 of exposure in terms of mg/kg bw doses associated with field exposures are not available.
9 Nonetheless and as discussed in Section 4.1.2.4.4, field and field simulation studies suggest that
10 risks associated with contact exposures for bees can be reduced if contact is delayed for a period
11 of at least 3 hours following application. These observations cannot be used to develop a formal
12 exposure assessment but are discussed further in the risk characterization for terrestrial
13 invertebrates (Section 4.4.2.4.1).

14 **4.2.4. Terrestrial Plants**

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

23 **4.2.5. Aquatic Organisms**

24 An assessment of the effects of spinosad on aquatic organisms is based on estimated
25 concentrations of spinosad in water that are identical to those used in the human health risk
26 assessment (Section 3.2.3.4.6) and the risk assessment for terrestrial vertebrates (Section
27 4.2.2.4). The water contamination rates are summarized in Table 14, and the application of these
28 rates to estimating expected concentrations of spinosad in water are discussed in Section
29 3.2.3.4.6.

30

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 [$370 \text{ mg/kg bw} \div 0.041 \text{ mg/kg bw} \approx 9024$] and more sensitive than birds by a factor of over nearly 5000 [$200 \text{ mg/kg bw} \div 0.041 \text{ mg/kg bw} \approx 4878$]. 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 [$0.498 \text{ mg a.i./L} \div 0.0005 \text{ mg a.i./L} = 996$]. Based on chronic NOAECs for tolerant species, aquatic invertebrates are more sensitive than fish by a factor of about 14 [$1.15 \text{ mg a.i./L} \div 0.0842 \text{ mg a.i./L} \approx 13.658$]. 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 $2.49 \text{ mg/kg bw/day}$ (U.S. EPA/OPP/HED 2011a, MRID 47011901). Following standard practice in Forest

1 Service risk assessments, this chronic NOAEL is used as the basis for the chronic risk
2 characterization in canids. As summarized in Appendix 1, Table A1-2, the use of the NOAEL
3 2.49 mg/kg bw/day for spinetoram is supported by a chronic NOAEL of 2.68 mg/kg/day for
4 spinosad exposure in dogs of (Harada 1995, MRID 43701504).

5
6 The ecological risk assessment attempts to identify subgroups of organisms that may display
7 greater or lesser sensitivity to a particular pesticide. These differences may be based on
8 allometric scaling (e.g., Sample and Arenal 1999) or differences in physiology. As discussed in
9 Section 3.1.5 and illustrated in Figure 5, dogs appear to be somewhat more sensitive than rodents
10 to spinosad; furthermore, the greater sensitivity of dogs is reflected in the toxicity values for
11 human health documented in U.S. EPA/OPP/HED (2011a) and used in the current Forest Service
12 risk assessment. In the recent preliminary ecological assessment associated with the registration
13 review of spinetoram, the acute LD₅₀ of 3738 mg/kg bw is the lowest acute oral toxicity value
14 cited (U.S. EPA/OPP/EFED 2011a, Table 11, pp. 27). As discussed in Section 3.1.4.1 and
15 summarized in Appendix 1, Table A1-1, this is the lowest definitive LD₅₀ value for spinosad
16 (MRID 43414515). The Forest Service prefers to use NOAELs rather than LD₅₀ values for risk
17 characterization. In the absence of a NOAEL from this study, the LD₅₀ is divided by 10 and
18 rounded to two significant figures to approximate a NOAEL of 370 mg/kg bw. This approach to
19 estimating a NOAEL from an LD₅₀ is consistent with EPA's variable level-of-concern method,
20 as detailed in SERA (2014a, Section 4.3.2).

21
22 For longer-term exposures in non-canid mammals, U.S. EPA/OPP/EFED (2011a, Table 11, pp.
23 28) identifies a NOAEL of 10 mg/kg bw/day citing MRID 43701506. As summarized in
24 Appendix 1, Table A1-3, this MRID refers to a standard reproduction study in rats. This
25 NOAEL is supported by a NOAEL of 9.5 mg/kg/day in male rats from a standard chronic
26 toxicity study (Bond et al. 1995b, MRIDs 43701507 and 43710503). For the current Forest
27 Service risk assessment, the NOAEL of 10 mg/kg bw/day is adopted from U.S. EPA/OPP/EFED
28 (2011a). A previous EPA ecological risk assessments identifies a dietary NOAEL of >1100 ppm
29 for MRID 43701506 (U.S. EPA/OPP/EFED 2009a, p. 40). While this information is provided
30 for the sake of completeness, the more recent EPA documents cited above, reclassify the
31 NOAEL as 10 mg/kg bw/day corresponding to a dietary concentration of 200 ppm.

32 **4.3.2.2. Birds**

33 As with mammals, Forest Service risk assessments generally defer to the U.S. EPA/OPP on
34 study selection, unless there is a compelling reason to do otherwise. For characterizing risks to
35 birds, U.S. EPA/OPP/EFED (2010a, Table 8, p. 25) uses an indefinite gavage LC₅₀ of >1333
36 mg/kg bw in quail (Murray et al. 1992b, MRID 43414529, as summarized in Appendix 2, Table
37 A2-1) to characterize risks associated with acute exposures. Based on the DER for this study,
38 the NOAEL for quail is 200 mg/kg bw with a LOAEL of 500 mg/kg bw based on ataxia.
39 Following the preference by the Forest Service to use NOAELs for risk characterization, the
40 NOAEL of 200 mg/kg bw is used to characterize risks associated with acute exposures of birds
41 to spinosad.

42
43 For chronic exposures, U.S. EPA/OPP/EFED (2010a, Table 8, p. 25) uses a dietary NOAEC of
44 550 mg/kg diet from standard reproduction studies in quail (Beavers et al. 1994a, MRID
45 43414533) and mallards (Beavers et al. 1994b, MRID 43414532). As summarized in Appendix
46 2, Table A2-2 of the current risk assessment, both of these studies yield LOAELs of 1100 mg/kg

1 diet based on embryotoxicity and decreased survival of offspring. As discussed in Section
2 4.2.2.3, the exposure assessment for birds is based on doses in units of mg/kg bw derived from
3 food consumption estimates based on the concentration of the pesticide in food, the caloric
4 values of different foods, and the caloric requirements of birds. Thus, the dietary NOAECs in
5 units of mg/kg food are converted to doses in units of mg/kg bw. For both mallards and quail,
6 dietary concentrations (mg/kg diet) are converted to mg/kg bw/day doses using a food
7 consumption factor of 0.07 kg food/kg bw based on reproduction studies in quail and mallards
8 (SERA 2007b). Using this food consumption factor, the dietary NOAEC of 550 mg a.i./kg diet
9 corresponds to a dose of 38.5 mg/kg bw [550 mg/kg food x 0.07 kg food/kg bw].

10 **4.3.2.3. Reptiles and Amphibians (Terrestrial Phase)**

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

13 **4.3.2.4. Terrestrial Invertebrates**

14 **4.3.2.4.1. Contact Toxicity (bees)**

15 The effects of direct spray or spray drift to terrestrial insects are typically assessed using the
16 results of contact toxicity studies—i.e., studies in which the pesticide is applied by pipette to the
17 insect. As discussed in Section 4.1.2.4.3, summarized in Table 19, and illustrated in Figures 6
18 and 7, contact toxicity assays are available on several species of terrestrial invertebrates. The
19 LD₅₀ values from the available studies are highly variable ranging from 0.025 mg/kg bw for the
20 honey bee to 65 mg/kg bw for a bumblebee (*Bombus terrestris*). This wide range spans a factor
21 of 2600. Even within the assays on honeybees, the reported LD₅₀ values range from 0.025 to 8.5
22 mg/kg bw, spanning a factor of about 340.

23
24 Consistent with the most recent EPA ecological risk assessment (U.S. EPA/OPP 2010a, p. 39),
25 the lowest LD₅₀ of 0.0029 mg/bee is from the study by Hoxter et al. (1992, MRID 43414547).
26 The DER for this study indicates a NOAEL of 0.0016 µg a.i./bee, based on treatment-related
27 mortality and signs of toxicity seen at higher doses. Taking a body weight of 116 mg for the
28 honeybee (Table 18), this NOAEL is equivalent to a dose of about 0.014 mg/kg bw [0.0016 µg ÷
29 0.116 g ≈ 0.01379 µg/g bw (mg/kg bw)].

30
31 While the NOAEL of 0.014 mg/kg bw is used in the EXCEL workbooks (Attachments 1 and 2)
32 to calculate HQs for bees following contact exposure, the wide range of toxicity values discussed
33 above is considered further in the risk characterization for terrestrial invertebrates (Section
34 4.4.2.4.1).

35 **4.3.2.4.2. Oral Toxicity (bees)**

36 The U.S. EPA risk assessments on spinosad do not explicitly derive toxicity values for oral
37 exposures in bees and note that oral toxicity data for the honey bee was not submitted to the EPA
38 (U.S. EPA/OPP/EFED 2011a, Table 14, p. 60). As summarized in Table 17 and detailed further
39 in Appendix 3, Table A3-1, several acute oral LD₅₀ values for honeybees are available in the
40 open literature. The oral LD₅₀ values in honeybees technical grade spinosad and spinosad
41 formulations are remarkably consistent with a range from 0.41 to 0.54 mg/kg bw. As discussed
42 in Section 4.3.2.1, the Forest Service prefers to use NOAELs rather than LD₅₀ values for risk
43 characterization. Adopting the EPA's variable level of concern method (SERA 2014a, Section

1 4.3.2), NOAELs may be approximated by dividing an acute LD₅₀ by a factor of 10. In the
2 absence of an acute NOAEL for honeybees, the lowest acute oral LD₅₀ of 0.41 mg/kg bw
3 (Carvalho et al. 2013) could be divided by 10 to approximate an acute NOAEL of 0.041 mg/kg
4 bw.

5
6 Based on the contact toxicity data discussed in the previous section, dividing the oral NOAEL by
7 a factor of 10 might be viewed as overly conservative. The contact assay by Hoxter et al. (1992,
8 MRID 43414547) yields an LD₅₀ of 0.029 mg/kg bw with an NOAEC of 0.014 mg/kg bw. The
9 ratio of the NOAEL to the LD₅₀ is 0.56 [0.014 mg/kg bw ÷ 0.029 mg/kg bw ≈ 0.013793].

10 Taking the acute oral LD₅₀ of 0.41 mg/kg bw, the ratio of the contact NOAEL to contact LD₅₀
11 could be used to estimate an oral NOAEL of 0.23 mg/kg bw [0.41 mg/kg bw x 0.56 = 0.2296
12 mg/kg bw].

13
14 Another alternative to using the standard factor of 10 to estimate an NOAEL from an acute LD₅₀
15 could be based on the study by Barbosa et al. (2015). As discussed in Section 4.1.2.4.2, a dose
16 of about 0.056 mg a.i./kg bw/day was associated with about 50% mortality in *Melipona*
17 *quadrifasciata*, a species of stingless bee. The NOAEL for gross signs of toxicity (i.e., decreases
18 in survival and pupal body weights as well as increases in the incidence of malformations) was
19 about 0.012 mg a.i./kg bw/day. Based on this relationship, a factor of about 5 might be used to
20 approximate the NOAEL [0.059 mg a.i./kg bw/day ÷ 0.012 mg a.i./kg bw/day ≈ 4.917].

21
22 A potential concern with using a factor of 5 based on gross signs of toxicity is the much lower
23 NOAEC of 0.00059 mg/kg bw/day reported by Barbosa et al. (2015) based on abnormal walking
24 activity in newly emerged adults. This NOAEL is a factor of 100 below the approximate LD₅₀
25 [0.059 mg a.i./kg bw/day ÷ 0.00059 mg/kg bw/day = 100]. This relationship would suggest that
26 the standard factor of 10 would be insufficient to estimate the NOAEL. The use of a factor of
27 100, however, could be viewed as overly conservative. As noted in the discussion by Barbosa et
28 al. (2015):

29
30 *To confirm the differences [in walking behavior] observed in the present study*
31 *with bioinsecticide-exposed stingless bees, more complex experimental setups,*
32 *including semifield and field studies, need to be performed. Such setups will allow*
33 *the assessment of more complex behaviors, such as foraging, which are very*
34 *important for colony survival.*

35
36 In other words, the concerns with pollinators are primarily focused on colony health. In the
37 absence of confirming studies that would demonstrate an impact on colony health, the
38 quantitative use of the very low NOAEL for walking behavior does not seem justified.

39
40 Given the above considerations, the current Forest Service risk assessment adopts the standard
41 approach and estimates an NOAEL of 0.041 mg a.i./kg bw for the honeybee by dividing the
42 lowest LD₅₀ of 0.41 mg a.i./kg bw by a factor of 10. Reservations with this approach are
43 considered further in the risk characterization (Section 4.4.2.4.3, Contaminated Nectar).

44 **4.3.2.4.3. Oral Toxicity (phytophagous insects)**

45 As summarized in Table 17 and discussed above, the acute oral LD₅₀ values for honeybees are
46 remarkably consistent, spanning a narrow range of 0.41 to 0.54 mg/kg bw with an average value

1 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
2 summarized in Table 17, an LD₅₀ of 0.23 mg/kg bw is available for spinosyn A in a
3 phytophagous insect, the larvae of *Heliothis virescens* (tobacco budworm). This LD₅₀, which is
4 lower than the estimated NOAEC of 0.3 mg/kg bw for the honeybee (Section 4.3.2.4.2), involved
5 an injection exposure rather than an oral exposure. Because of this route of exposure as well as
6 the nature of agent (i.e., spinosyn A rather than spinosad), the application of the LD₅₀ to 0.23
7 mg/kg bw to the dose-response assessment for phytophagous insects seems questionable. On the
8 other hand, as summarized in Table 17, lower oral LD₅₀ values are available for *Bombus*
9 *terrestris* (LD₅₀ = 0.13 mg/kg bw) and a stingless bee, *Melipona quadrifasciata* (LD₅₀ = 0.15
10 mg/kg bw). While these species are not phytophagous insects, the LD₅₀ values support the
11 assessment that in terms of oral exposure to spinosad, honeybees are not the most sensitive insect
12 species.

13
14 For the current risk assessment, the lowest LD₅₀ (i.e., 0.13 mg/kg bw for *Bombus terrestris*) is
15 used as the basis for the dose response assessment for phytophagous insects. This approach is
16 supported by the more extensive data on contact toxicity in insects (as illustrated in Figure 7 and
17 discussed in Section 4.1.2.4.3.2) indicating that there are no substantial differences in sensitivity
18 among Hymenoptera, Diptera, and Lepidoptera (an order of insects which are largely
19 phytophagous). As with the approach used in estimating an oral NOAEL for honeybees (Section
20 4.3.2.4.2), the LD₅₀ of 0.13 mg/kg bw for *Bombus terrestris* is multiplied by a factor of 0.64 and
21 rounded to one significant place to estimate a NOAEC of 0.08 mg a.i./kg bw [0.13 mg/kg bw x
22 0.64 = 0.0832 mg/kg bw] for phytophagous insects.

23
24 The use of the 0.64 ratio derived from the contact toxicity data in the honeybee (Section
25 4.3.2.4.2.) is less well supported than the application of this factor to a phytophagous insect,
26 because this application involves both route-to-route and species-to-species extrapolation. As
27 discussed further in Section 4.4.2.4.2, the use of the 0.64 ratio rather than the default 0.1 ratio has
28 no impact on the qualitative risk characterization for phytophagous insects—i.e., all HQs exceed
29 the level of concern even at the lower bounds of exposures.

30 **4.3.2.4.4. Soil Exposures (earthworms)**

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

37 **4.3.2.5. Terrestrial Plants (Macrophytes)**

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

41 **4.3.2.6. Terrestrial Microorganisms**

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

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

3 **4.3.3. Aquatic Organisms**

4 **4.3.3.1. Fish**

5 **4.3.3.1.1. Acute Toxicity**

6 As discussed in Section 4.1.3.1.1 and summarized in Appendix 5, Table A5-1, the available
7 literature on spinosad includes several standard LC₅₀ values in fish which the EPA uses to
8 classify spinosad as slightly to moderately toxic to fish. While LC₅₀ values are used directly in
9 EPA risk assessments, the Forest Service prefers to use NOAECs in the dose-response
10 assessment (SERA 2014a, Section 4.3). As noted in Appendix 5, Table A5-1, Data Evaluation
11 Records (DERs as discussed in Section 1.2) are available for two species of fish, and the
12 NOAECs for sublethal effects range from 1.8 mg a.i./L (sheepshead minnow with a
13 corresponding LC₅₀ of 7.87 mg a.i./L from York 1993) to 2.1 mg a.i./L (bluegill sunfish with a
14 corresponding LC₅₀ of 5.94 mg a.i./L from the study by Newsted and Brock 1992). While these
15 are the best documented sublethal NOAECs, the LC₅₀ values from these studies encompass a
16 narrow range.

17
18 Of the reasonably well-documented LC₅₀ values in fish, the lowest LC₅₀ is 4.99 mg a.i./L in carp
19 from the review by Cleveland et al. (2002b). The DER for the study by York (1993) yields the
20 lowest ratio of NOAEC to LC₅₀—i.e., about 0.23 [1.8 mg a.i./L ÷ 7.87 mg a.i./L ≈ 0.2287]. This
21 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
22 mg a.i./L], which is used to characterize risks in sensitive species of fish.

23
24 The highest definitive LC₅₀ in fish is 30.0 mg a.i./L in rainbow trout from MRID 43444103, as
25 summarized in both U.S. EPA/OPP/EFED (2011a) and Cleveland et al. (2002b). From the 0.23
26 ratio discussed above, the NOAEC for trout is estimated at 6.9 mg a.i./L [30 mg a.i./L x 0.23],
27 which is supported by the NOAEC (normal swimming behavior) of 10 mg a.i./L in coho salmon
28 from the study by Deardorff and Stark (2009). Deardorff and Stark (2009) also report an
29 indefinite LC₅₀ of >500 mg a.i./L in coho salmon. The somewhat lower estimated NOAEC of
30 6.9 mg a.i./L is used in the current risk assessment for tolerant species of fish.

31 **4.3.3.1.2. Chronic Toxicity**

32 As discussed in Section 4.1.3.1.2 and summarized in Appendix 5, Table A5-2, only two early
33 life-stage studies were submitted to and accepted by the EPA: the standard early life stage
34 studies in trout (Weinberg et al. 1993, MRID 43414541) and sheepshead minnow (MRID
35 44420601). The study in trout yields an NOAEC of 0.498 mg a.i./L, which is used to
36 characterize risks associated with longer-term exposures in sensitive species of fish. The EPA
37 uses this chronic NOAEC in trout to characterize longer-term risks to fish (U.S. EPA/OPP/EFED
38 2009a, p. 39). The early-life stage study in sheepshead minnow yields an NOAEC of 1.15 mg
39 a.i./L, which is used to characterize risks associated with longer-term exposures in tolerant
40 species of fish.

1 One reservation with this relatively standard and uncomplicated dose-response assessment for
2 longer-term exposures of fish to spinosad involves the presumed sensitivity of trout. As
3 discussed in the previous section, trout appear to be tolerant species in terms of acute exposures
4 with an acute LC₅₀ of 30 mg a.i./L and an estimated NOAEC of 6.9 mg a.i./L. The most
5 sensitive species of fish in terms of acute toxicity is carp with a LC₅₀ of 4.99 mg a.i./L and an
6 estimated NOAEC of 1.1 mg a.i./L. In terms of acute toxicity, sheepshead minnow have an
7 intermediate LC₅₀ of 7.87 mg a.i./L and an experimental NOAEC of 1.8 mg a.i./L.

8
9 The lack of correspondence between sensitivities of different species of fish in acute and longer-
10 term exposures is noted for the sake of clarity and transparency. While several different
11 approaches using acute-to-chronic ratios could be used to estimate lower NOAECs for fish (NAS
12 2013), these approaches are typically reserved for addressing a lack of data rather than as an
13 alternative to experimental data. From a practical perspective, reasonable applications of acute-
14 to-chronic ratio methods would not have an impact on the risk characterization for longer-term
15 exposures of fish to spinosad. As discussed further in Section 4.4.3.1, the HQs for fish are
16 substantially below the level of concern.

17 **4.3.3.2. Amphibians (Aquatic Phase)**

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

20 **4.3.3.3. Aquatic Invertebrates**

21 **4.3.3.3.1. Acute Toxicity**

22 **4.3.3.3.1.1. Sensitive Species**

23 As discussed in Section 4.1.3.3.1 and summarized in Table 25, data regarding the acute toxicity
24 data of spinosad to aquatic invertebrates are more than ample. As with other groups of
25 organisms, Forest Service risk assessments typically defer to EPA in terms of study selection for
26 dose-response assessments, unless there is a compelling reason to do otherwise. In the case of
27 spinosad, EPA considers some but not all of the relevant open literature (i.e., U.S.
28 EPA/OPP/EFED 2011a, pp. 72-73). Specifically, the EPA does not consider or cite the study by
29 Deardorff and Stark (2009) on three species of cladocerans. As summarized in Table 25, the
30 study by Deardorff and Stark (2009) notes a 48-hour LC₅₀ of 0.0018 mg a.i./L in a bioassay of a
31 Success[®] formulation in *Ceriodaphnia dubia* [Cladocera: Daphniidae]. This LC₅₀ is supported
32 by several additional LC₅₀ values in the range of 0.002 to 0.009 mg a.i./L in two families of
33 Diptera—i.e., midge larvae [Diptera: Chironomidae, LC₅₀ = 0.009 mg a.i./L], mosquito larvae
34 [Diptera: Culicidae, LC₅₀ = 0.002 mg a.i./L in *Anopheles stephensi*, LC₅₀ = 0.0032 mg a.i./L in
35 *Culex pipiens*, and LC₅₀ = 0.007 in *Aedes aegypti*] (see Table 25 for details). For risk
36 characterization in aquatic invertebrates, U.S. EPA/OPP/EFED (2009a, p. 39) uses the EC₅₀ of
37 14 mg a.i./L in *Daphnia magna* from the study by Milazzo et al. (1994, MRID 43574502).

38
39 While the study from Deardorff and Stark (2009) is not specifically reviewed by EPA, it appears
40 to have been well conducted and documented, is published in a peer reviewed journal (Journal of
41 Environmental Science and Health, Part B), was conducted at an academic institution in the
42 United States (Washington State University, Puyallup Research and Extension Center), and was
43 funded by NOAA (National Oceanic and Atmospheric Administration). As discussed in Section
44 4.1.3.3.1, however, the study by Deardorff and Stark (2009) was conducted using a formulation

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

10
11 Another concern with the use of formulation data involves environmental partitioning—i.e., the
12 separation of inerts from the active ingredient over time due to differences in environmental fate
13 characteristics of the components in the formulation, including the active ingredient. This
14 consideration, however, affects the consideration of longer-term effects rather than acute effects
15 (e.g., NAS 2013, pp. 121-122).

16
17 The above considerations constitute a compelling basis for differing from rather than deferring to
18 the EPA. The dose-response assessment for sensitive species of aquatic invertebrates could be
19 based on the LC₅₀ of 0.0018 mg a.i./L in *Ceriodaphnia dubia*. Typically, a Forest Service risk
20 assessment would divide the EC₅₀ by a factor of 20 to approximate an acute NOAEL of 0.00009
21 mg a.i./L or 0.09 μ g a.i./L [0.0018 mg/L \div 20] (SERA 2014a, Section 4.3.2, pp. 98-99). As
22 discussed further in Section 4.3.3.3.2, however, a subsequent study by Deardorff and Stark
23 (2011) determined a chronic NOAEC for *Ceriodaphnia dubia* of 0.5 μ g a.i./L (0.0005 μ g a.i./L).
24 It would not be sensible to derive an acute NOAEC that is below the chronic NOAEC. As a
25 possible alternative, the study in *Daphnia magna* used by EPA (i.e., Milazzo et al. 1994, MRID
26 43414537/43574502) report an EC₅₀ of 14 mg a.i./L with a corresponding NOAEC of 0.883 mg
27 a.i./L. The ratio of these values is about 0.063 [0.883 \div 14 \approx 0.06307]. When multiplied by this
28 ration, the LC₅₀ of 0.0018 mg a.i./L in *Ceriodaphnia dubia* would estimate a NOAEC of about
29 0.00011 mg a.i./L or 0.11 μ g a.i./L [0.0018 mg a.i./L \times 0.063 \approx 0.0001134 mg a.i./L]. This
30 value is also below the chronic NOAEC 0.5 μ g a.i./L reported by Deardorff and Stark (2011).
31 Thus, in the absence of a viable alternative, the chronic NOAEC of 0.0005 mg a.i./L for
32 *Ceriodaphnia dubia* is applied to acute exposures.

33 34 **4.3.3.3.1.2. Tolerant Species**

35 As summarized in Table 25, toxicity assays with aquatic invertebrates yielding EC₅₀ values in
36 excess of 1 mg a.i./L include three bioassays with *Daphnia magna* (Cleveland et al. 2002b;
37 European Commission 2006; Milazzo et al. 1994, MRID 43574502), one bioassay with *Aedes*
38 *aegypti* (Kovendan et al. 2012), one bioassay in an amphipod (MRID 47702901), and a bioassay
39 in shrimp (Cleveland et al. 2002b). As discussed in Section 4.1.3.3.1, all of the studies yielding
40 EC₅₀ values greater than 1 mg a.i./L involve technical grade spinosad; formulations of spinosad
41 appear to be much more toxic. As discussed in the previous section on sensitive species of
42 aquatic invertebrates, the greater toxicity of spinosad formulations is relevant to the dose-
43 response assessment for acute exposures.

44
45 As discussed in Section 4.1.3.3.1.2, several studies conducted in various species of mosquitos
46 note substantial resistance to spinosad formulations. As with terrestrial insects (as summarized

1 in Table 24), very high resistance factors were observed in mosquitos following artificial
2 selection pressure—e.g., the LC₅₀ of 693.5 mg a.i./L in *Culex quinquefasciatus* (Su and Chen
3 2014b). These toxicity values are not considered for the dose-response assessment because the
4 type of artificial selection pressure is not relevant to environmental exposures. Nevertheless, as
5 summarized in Table 25, Su and Chen (2014b) report LC₅₀ values of 0.196 to 0.460 mg a.i./L for
6 a Natular[®] formulation in 3rd instar larvae of *Culex quinquefasciatus* from a wild population with
7 no artificial selection pressure. A similar range of LC₅₀ values—i.e., 0.234 to 0.424 mg a.i./L—
8 is reported for a laboratory population of *Culex quinquefasciatus* with no prior exposure to
9 spinosad. Consequently, it seems reasonable to use the upper bound LC₅₀ of 0.460 mg a.i./L to
10 represent relevant tolerant species/populations of aquatic invertebrates. Dividing this EC₅₀ by a
11 factor of 20 (SERA 2014a, Section 4.3.2, pp. 98-99) results in a NOAEL of 0.023 mg a.i./L
12 [0.460 mg a.i./L ÷ 20].

13 **4.3.3.3.2. Chronic Toxicity**

14 The dose-response assessment for longer-term exposures to spinosad in aquatic invertebrates is
15 relatively straightforward. As summarized in Table 26 and discussed in Section 4.1.3.3.2.1,
16 daphnids and midges appear to be sensitive groups of aquatic invertebrates with most of the
17 NOAECs spanning a relatively narrow range of 0.5 to 1.6 µg a.i./L. The only exception is the
18 NOAEC of 8 µg a.i./L for a static renewal reproduction study in *Daphnia magna* reported by the
19 European Commission (2006). The NOAEC of 1.2 µg a.i./L for a flow-through reproduction
20 study in *Daphnia magna*, also reported in the review by European Commission (2006), is
21 consistent with other NOAECs for daphnids and midges.
22

23 As discussed in Section 4.1.3.3.2.1, the LOAEC of 129 µg a.i./L in daphnids from the study by
24 Stark (2005), which is summarized in Table 26, is not a reproduction study, and the high
25 LOAEC simply reflects the study design—i.e., a single concentration substantially greater than
26 the 48-hour EC₅₀ with a nominal 10-day period of exposure.
27

28 Unlike the case with acute exposures of aquatic invertebrates, formulations of spinosad do not
29 appear to be substantially more toxic than technical grade spinosad. This assessment is based
30 primarily on the chronic bioassays in *Daphnia magna* using technical grade spinosad (MRID
31 43848801, NOAEC = 0.62 µg a.i./L, LOAEC = 1.2 µg a.i./L) and *Ceriodaphnia dubia* using a
32 Success[®] formulation (Deardorff and Stark 2011, NOAEC 0.5 µg a.i./L, LOAEC = 1 µg a.i./L).
33

34 For the dose-response assessment of sensitive species of aquatic invertebrates involving longer-
35 term exposures, the chronic NOAEC of 0.5 µg a.i./L in *Ceriodaphnia dubia* is used.
36

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

41 **4.3.3.4. Aquatic Plants**

42 **4.3.3.4.1. Algae**

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

1 freshwater diatom, *Navicula pelliculosa* with an EC₅₀ of 0.09 mg a.i./L and an NOAEC of 0.05
2 mg a.i./L (MRID 43414543). The NOAEC is used to characterize risks in sensitive species of
3 algae.

4
5 In terms of both EC₅₀ and NOAEC values, the most tolerant species is a green alga, *Selenastrum*
6 *capricornutum*, with an indefinite EC₅₀ of >105.5 mg/L and a corresponding NOAEC of 4.3 mg
7 a.i./L (Cleveland et al. 2002b). The NOAEC of 4.3 mg a.i./L is used to characterize risks in
8 tolerant species of algae.

9 **4.3.3.4.2. Aquatic Macrophytes**

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

14

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

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

3
4 Aquatic invertebrates, particularly sensitive species, could be at substantial risk following the
5 application of spinosad in areas where the potential for water contamination is high, including
6 areas with moderate to heavy rainfall. In arid areas, particularly areas with predominantly loam
7 or sand soil textures, adverse effects on even sensitive species of aquatic invertebrates might not
8 be observed. Given the variability in the estimated concentrations of spinosad in water, no
9 general risk characterization for aquatic invertebrates is justified. In any site-specific application
10 of spinosad, the risks will vary substantially with local conditions. Given the highly variable
11 results from the generic water modeling used in the current risk assessment and the substantial
12 impact that this variability has on the risk characterization for aquatic invertebrates, site-specific
13 efforts to estimate surface water concentrations of spinosad might be justified, particularly in
14 areas with moderate to heavy rainfall.

15
16 Vertebrates are less sensitive than invertebrates to spinosad. Nonetheless, foliar applications of
17 spinosad could result in exposure levels that exceed the level of concern for some terrestrial
18 mammals (longer-term exposures only) and birds (both acute and longer-term). For non-
19 accidental exposure scenarios, risks to mammals and birds are associated with the consumption
20 of contaminated vegetation, and risks are greatest for smaller animals consuming contaminated
21 grasses or food items with spinosad concentrations comparable to those associated with
22 contaminated grasses. The only HQ for accidental exposure scenario for terrestrial vertebrates
23 that exceeds the level of concern is the upper bound HQ for a canid consuming contaminated
24 fish. Except for an accidental spill scenario, risks to fish and aquatic vegetation appear to be
25 insubstantial.

26
27 The risk characterization for spinosad focuses on the potential for direct toxic effects.
28 Nonetheless, there is a potential for secondary or indirect effects in virtually all groups of
29 nontarget organisms. Terrestrial applications of any effective insecticide, including spinosad, are
30 likely to alter insect and other invertebrate populations within the treatment area. This alteration
31 could have indirect effects on terrestrial or aquatic animals and plants, including changes in food
32 availability, predation, and habitat quality. These indirect effects may be beneficial to some
33 species and detrimental to others; moreover, the magnitude of indirect effects is likely to vary
34 over time.

35 **4.4.2. Terrestrial Organisms**

36 **4.4.2.1. Mammals**

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

42
43 Only one upper bound HQ for accidental exposures exceeds the level of concern—i.e., an upper
44 bound HQ of 17 for a canid consuming contaminated fish following an accidental spill. As
45 discussed in Section 4.3.2.1, canids are considered a sensitive subgroup of mammals with a

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

9
10 In terms of chronic exposures, scenarios for the consumption of contaminated vegetation exceed
11 the level of concern for both a single application (Attachment 1, upper bound HQs of 1.7 to 7).
12 For two applications (Attachment 2), the upper bound HQs exceed the level of concerns for
13 contaminated vegetation (HQs of 1.5 to 14) and contaminated fruit (an upper bound HQ of 1.2
14 for a small mammal. In all cases, the HQs are highest for small mammals.

15
16 The HQs for mammals are based on the assumption that 100% of the diet is contaminated
17 (SERA 2014a, Section 4.2.2.3). This assumption may be unrealistic for some acute exposures
18 and will probably be a rare event in terms of chronic exposures, at least for larger mammals (i.e.,
19 larger animals may move in and out of the treated areas). The impact of a limited consumption
20 of contaminated vegetation based on less than 100% of the diet as contaminated is not
21 considered quantitatively in the current risk assessment. Nonetheless, this consideration could be
22 justified at least for some species in site-specific applications of spinosad.

23 **4.4.2.2. Birds**

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

29
30 Several acute non-accidental and longer-term exposure scenarios for the consumption of
31 contaminated vegetation exceed the level of concern for a small (10 g) bird but not for a larger (4
32 kg) bird. This pattern is similar to the pattern observed in mammals (Section 4.1.2.1) and
33 reflects the greater food consumption of smaller birds relative to larger birds.

34
35 For acute exposures following a single application, the exceedances in the upper bound HQs are
36 minor—i.e., an upper bound of 1.1 for contaminated broadleaf foliage and 1.9 for the
37 consumption of short grass. For two applications, the exceedances in the upper bound HQs are
38 about twice as high—i.e., an HQ of 2 for broadleaf vegetation and 4 for short grass. As
39 discussed in Section 4.3.2.2, the acute LOAEC for birds is a factor of 2.5 higher than NOAEC
40 (i.e., 500 mg/kg bw vs 200 mg/kg bw from the acute gavage study). Thus, the HQ of 4 for a
41 small bird suggests that signs of toxicity might be observed. On the one hand, small birds
42 typically do not consume large amounts of grasses in the vegetative stage; on the other hand,
43 many birds consume significant amounts of grass seeds (USDA/NRCS 1999). Thus, concern for
44 the scenario involving the consumption of contaminated grasses by small birds may be most
45 relevant to contaminated grasses with seeds.

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

16 **4.4.2.3. Reptiles and Amphibians (Terrestrial Phase)**

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

19 **4.4.2.4. Terrestrial Invertebrates**

20 **4.4.2.4.1. Direct Spray**

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

23
24 Spinosad is an effective insecticide, and the direct spray of a bee at an application rate of 0.225
25 lb a.i./acre leads to an HQ of 1103. This HQ is associated with a dose of about 15.4 mg/kg bw,
26 which is above the lowest topical LD₅₀ for bees of 0.025 mg/kg bw (Hoxter et al. 1992) by a
27 factor of over 600 [15.4 mg/kg bw ÷ 0.025 mg/kg bw = 616]. As summarized in Table 19 and
28 discussed in Section 4.1.2.4.3, there is a wide range of contact LD₅₀ values for bees. Based on
29 the highest estimated LD₅₀ for honeybees of 8.5 mg a.i./kg bw (Mayes et al. 2003), the direct
30 spray exposure is higher than the LD₅₀ by a factor of about 2 [15.4 mg/kg bw ÷ 8.4 mg/kg bw ≈
31 1.8333...]. These HQs suggest that the direct spray of a honeybee with spinosad at the
32 application rate proposed by the Forest Service would be associated with substantial mortality in
33 even tolerant populations of bees. As also summarized in Table 19, most other insects on which
34 data are available, including other Hymenoptera, Diptera, and Lepidoptera, have contact LD₅₀
35 values in the range of 0.5 to 2 mg a.i./kg bw. While exposure assessments are not quantified for
36 these other groups of insects, it seems likely that they would be adversely affected by direct
37 spray. By definition, this severe risk characterization for terrestrial insects is probably applicable
38 to most insecticides applied at effective application rates.

39
40 The only noteworthy exception may involve the bumblebee. Based on the LD₅₀ of about 65
41 mg/kg bw in *Bombus terrestris* estimated from data in the review by Mayes et al. 2003, it is not
42 clear that all species of bumblebees would be adversely affected by direct spray. Substantial
43 reservations with this speculation, however, involve the lack of confirming contact LD₅₀ values

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

3
4 As summarized in Worksheet G09, risks of contact exposures decrease substantially with
5 increasing distance from the application site and increasing foliar interception. These are
6 common observations with the application of any insecticide. Particularly for honeybees,
7 application timing may be another substantial factor in mitigating risks. As noted in Section
8 4.1.2.4.1, all of the product labels contain language that should reduce the acute exposure of bees
9 to spinosad during or shortly after application. In addition, as discussed in Section 4.1.2.4.4.2,
10 the review by Mayes et al. (2003) cites unpublished field studies indicating that no signs of overt
11 toxicity to bees were observed following evening applications of spinosad (i.e., when bees were
12 not foraging). These studies, however, appear to have been relatively short-term and may not
13 have accounted for the impact of exposures via foraging, which are discussed further in
14 Section 4.4.2.4.3.

15
16 Lastly, incidents involving bee mortality associated with spinosad applications are not indicated
17 in a review of incident reports to EPA. This reservation, however, is not viewed as a substantial
18 factor in the risk characterization. As noted in the most recent EPA document on spinosad
19 ...absence of reported incidents should not be construed as the absence of incidents (i.e., U.S.
20 EPA/OPP/EFED 2011a, pp. 39).

21 **4.4.2.4.2. Consumption of Contaminated Vegetation**

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

28
29 The specific HQs for phytophagous insects are summarized in Worksheet G08b of the EXCEL
30 workbooks that accompany this risk assessment. For a single application (Attachment 1), the
31 lower bound HQs range from 5 to 51 depending on the type of vegetation consumed. The upper
32 bound HQs range from over 90 to nearly 1500. For two applications (Attachment 2), the lower
33 bound HQs range from 6 to 54 and the upper bound HQs range from 175 to over 2800.

34
35 As discussed in Section 4.3.2.4.3, there are some uncertainties in the dose-response assessment
36 for phytophagous insects. The estimated NOAEC of 0.0832 mg/kg bw on which the HQs are
37 based is derived from an oral LD₅₀ of 0.13 mg/kg bw for *Bombus terrestris* rather than an
38 injection LD₅₀ of 0.23 mg/kg bw in the tobacco budworm. In addition, the approximation of the
39 NOAEC is based on an adjustment factor of 0.64 rather than the more standard factor of 0.1.
40 While these limitations are noted for the sake of transparency, they have no impact on the risk
41 characterization. As summarized in Worksheet G08a, the HQs for broadleaf vegetation and
42 small insects are based on doses of about 13 (2 to 69) mg/kg bw. The central estimate is higher
43 than the highest oral LD₅₀ for an insect (i.e., 2.7 mg/kg bw in the American cockroach as
44 summarized in Table 17) by a factor of about 5. Similar comparisons may be made for two
45 applications as well as other types of vegetation. In most cases, there is no doubt that an
46 application of spinosad to vegetation at a rate of 0.225 lb a.i./acre will be detrimental to

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

7
8 As summarized in Table 24 and discussed in Section 4.1.2.4.6, resistance factors of up to about
9 2,000,000 are noted in insect populations subject to artificial selection pressure. These extreme
10 resistance factors are probably not relevant to the risk characterization. Resistance factors of up
11 to about 7000, however, are documented in field populations in the absence of artificial selection
12 pressure. Even with the very high HQs discussed above for phytophagous insects, it seems
13 reasonable to believe that some populations of resistant phytophagous insects might not be
14 adversely affected. Again, however, this assessment is simply a restatement of the common
15 problem that insects may develop resistance to otherwise effective insecticides unless prudent
16 steps are taken (i.e., varying the types of insecticides applied) to minimize the development of
17 resistance. This approach is essentially the motivator for organizations such as the IRAC
18 Resistance Action Committee (IRAC 2016) as well as cautionary statements concerning
19 resistance on the labels for most insecticides including spinosad.

20 **4.4.2.4.3. Contaminated Nectar**

21 The HQs for foraging bees are summarized in Worksheet G10 for one application at a rate of
22 0.225 lb a.i./acre (Attachment 1) and two applications at the same rate but with a 6-day
23 application interval (Attachment 2). The HQs are 10 (5 to 22) for a single application and 15 (5
24 to 41) for two applications. As discussed in Section 4.3.2.4.2, the HQs are based on a NOAEC
25 in honeybees of 0.041 mg/kg bw estimated from an oral LD₅₀ of 0.41 mg/kg bw – i.e., the LD₅₀
26 is a factor of 10 above the estimated NOAEC. Thus, the ratio of the exposures to the LD₅₀ are 1
27 (0.5 to 2.2) for a single application and 1.5 (0.5 to 4.1) for two applications. Note that the
28 similarity in the lower bound values of the ratios is attributable to the lower bound of the
29 estimated half-life of spinosad in nectar (1.5 days) relative to the 6 day application interval (i.e.,
30 four half-lives.

31
32 Qualitatively, the risk characterization is unequivocal at the central estimates and upper bounds
33 of the estimated exposures. These exposures reach or exceed the LD₅₀; thus, they could be
34 associated with readily observable and perhaps substantial mortality in honeybees. At the lower
35 bound of HQs, the estimated exposures are approximately one-half of the LD₅₀. As discussed in
36 Section 4.3.2.4.2, doses associated with factors of 0.2 to 0.56 of the LD₅₀ might not be associated
37 with substantial or even observable rates of mortality. Nonetheless, as illustrated in the study by
38 Barbosa et al. (2015), sublethal signs of toxicity (altered patterns of movement) could occur at
39 doses substantially below the LD₅₀ as well as NOAECs for gross signs of toxicity. Whether or
40 not the sublethal effects on locomotion would be sufficiently severe to impact colony health is
41 unclear.

42
43 While the oral toxicity data on honeybees are reasonably complete and consistent (Section
44 4.3.2.4.2), there are major uncertainties in the exposure assessment (Section 4.2.3.3).
45 Specifically, there are no monitoring studies on the levels of spinosad in nectar; hence, the
46 concentrations are approximated using monitoring data for spinosad in pollen (Bailey et al. 2005)

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

5
6 As summarized in Table 22 and discussed in Section 4.1.2.4.4.2, the above risk characterization
7 for foraging honeybees has only limited support from the available field and field simulation
8 studies. At application rates in the range of about 0.2 lb a.i./acre, an increase in honeybee
9 mortality was observed; however, it is not clear that the increases were statistically significant
10 (Mayes et al. 2003, citing studies by Kirkland 1999 and Halsall 2002). At an application rate of
11 0.48 lb a.i./acre (roughly equivalent to two applications at 0.225 lb a.i./acre), increased mortality
12 was evident but not apparently statistically significant; nonetheless, a reduction was observed in
13 brood development (Mayes et al. 2003 citing Vinall 2000).

14
15 As also summarized in Table 22 and further detailed in Appendix 3, Table A3-4, several field
16 and field simulation studies indicate that no adverse effects were demonstrated in honeybees at
17 application rates of ≤ 0.16 lb a.i./acre. In reviewing these studies, Miles et al. (2011) note:

18
19 *Assessments performed up to 7 days after treatment made during bee activity*
20 *confirm the absence of mortality to foragers visiting treated flowers at 96 g*
21 *a.s./ha [≈ 0.085 lb a.i./acre]. Therefore, this exposure rate can be considered as a*
22 *threshold for immediate acute toxicity, but at which no long lasting acute toxicity*
23 *is expected at this application rate or higher.*

24 Miles et al. 2011, p. 113

25
26 In some respects, the suggestion that 0.085 lb a.i./acre may be a *threshold* for acute toxicity
27 seems overly conservative. As noted above and detailed in Appendix 3, field studies at or below
28 0.16 lb a.i./acre have not demonstrated acute adverse effects in honeybees. Nonetheless,
29 accepting the above estimate of 0.085 lb a.i./acre as a functional NOAEL based on a field study,
30 the application rate of 0.225 lb a.i./acre proposed by the Forest Service corresponds to an HQ of
31 about 3 [$0.225 \text{ lb a.i./acre} \div 0.085 \text{ lb a.i./acre} \approx 2.6$].

32
33 Substantial reservations with the available field studies involve the durations of exposure and
34 durations of observation. As summarized in Table A3-4, most of the available field studies
35 involve relatively brief periods of observation (1 to several days) that are focused more on the
36 impact of direct spray rather than exposures through foraging. The longest term field or field
37 simulation study is published in the paper by Morandin et al. (2005) and involved foraging by
38 bumblebees on artificial flowers over exposure periods of 3 to 5 weeks. Adverse effects noted in
39 this study included decreased levels of activity and trembling during foraging; moreover, these
40 effects occurred in exposures equivalent to application rates of about 0.07 lb a.i./acre, below the
41 presumptive NOAEL of 0.085 lb a.i./acre from Miles et al. (2011), as discussed above. As
42 discussed in Section 4.1.2.4.2, decreased activity was also observed in a species of stingless bee
43 at doses substantially below the LD_{50} in the study by Barbosa et al. (2015). The lack of longer-
44 term field studies, particularly studies involving colony overwintering, is a concern. As
45 discussed in the recent Forest Service risk assessment on imidacloprid (SERA 2015), longer-

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

3
4 The above risk characterization for foraging is focused on the honeybee because the exposure
5 assessment developed in the current risk assessment is based on published exposure assessment
6 methods for the honeybee – i.e., Alix and Vergnet (2007), Halm et al. (2006), and Rortais et al.
7 (2005) as detailed in Section 4.2.3.3.1. Nonetheless, as summarized in Table 17 and discussed in
8 Section 4.1.2.4.2, acute oral toxicity studies in a species of bumblebee (*Bombus terrestris* ,
9 Mayes et al. 2003) and a species of stingless bee (*Melipona quadrifasciata*, Tom et al. 2015)
10 suggest that these bees may be more susceptible than honeybees to spinosad. In addition, the
11 study by Barbosa et al. (2015) indicates that sublethal effects may occur in *Melipona*
12 *quadrifasciata* at doses substantially below those associated with gross signs of toxicity such as
13 mortality and reduced growth. While the studies on bees other than the honeybee are not used
14 quantitatively in the current risk assessment, these studies raise concern that adverse effects may
15 occur in other species of bees in addition to the honeybee.

16
17 The EPA did not conduct a risk assessment for foraging honeybees. In a recent ecological risk
18 assessment, the EPA notes: *Because spinosad is toxic to honeybees, risk is assumed* (U.S.
19 EPA/OPP/EFED 2009a, p. 48). On the other hand, the more recent ecological assessment of
20 spinosad in support of the registration review expresses little concern for the contamination of
21 pollen following foliar application: *Systemicity of spinosad into plant tissue, including possible*
22 *contamination of pollen and nectar related to pollinator health, does not appear to be a route of*
23 *concern considering that the majority of uses are foliar applications* (U.S. EPA/OPP/EFED
24 2011a). As summarized in Section 4.2.3.3.2, the study by Bailey et al. (2005), which provides
25 the residue data for spinosad in pollen, involved foliar application. While U.S. EPA/OPP/EFED
26 (2011a, p. 69) cites the study by Bailey et al. (2005), the residue data from the study are not
27 discussed in the EPA document. The analysis presented in the current Forest Service risk
28 assessment differs from the EPA’s assessment that the potential contamination of nectar and
29 pollen *...does not appear to be a route of concern*. As discussed in Section 2.2, spinosad is
30 labelled for and will be applied in broadcast applications. In broadcast applications, nontarget
31 plants that might be a source of nectar or pollen for honeybees may be contaminated. Based on
32 the exposure assessment and dose-response assessment in the current Forest Service risk
33 assessment, adverse effects including mortality in honeybees are plausible. While there are
34 substantial uncertainties with the exposure assessment presented in the current risk assessment,
35 these uncertainties do not negate concerns for potential effects on honeybees and other
36 pollinators following applications of spinosad.

37 **4.4.2.4.4. Soil Exposures**

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

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

4 **4.4.2.5. Terrestrial Plants**

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

13 **4.4.2.6. Terrestrial Microorganisms**

14 As with earthworms (Section 4.4.2.4.4), only limited information is available on the toxicity of
15 spinosad to terrestrial microorganisms. Based on studies briefly summarized in the the European
16 Commission (2006) review, adverse effects were not observed on nitrogen or carbon
17 mineralization by soil microorganisms at spinosad concentrations of 0.72 mg a.i./kg soil. This
18 NOAEC is above the maximum expected concentration of spinosad in soil (i.e., 0.19 mg a.i./kg
19 soil as discussed in Section 4.4.2.4.4) by a factor of about 4 [$0.72 \text{ mg/kg soil} \div 0.19 \text{ mg a.i./kg}$
20 $\text{soil} \approx 3.789$].

21 **4.4.3. Aquatic Organisms**

22 **4.4.3.1. Fish**

23 The HQs for fish are summarized in Worksheet G03 of Attachment 1 (one application) and
24 Attachment 2 (two applications). The risk characterization for fish is reasonably simple and
25 unequivocal. Based on expected levels of exposure (excluding accidental exposures), none the
26 HQs for fish exceeds the level of concern. The highest HQ is 0.07, the upper bound HQ for
27 sensitive species of fish based on acute exposure following two applications. This HQ is below
28 the level of concern by a factor of about 14 [$1 \div 0.07 \approx 14.286$]. Given the broad range of
29 conditions used to estimate expected concentrations of spinosad in surface water (Section
30 3.2.3.4.3), direct toxic effects on fish following applications anticipated in Forest Service
31 programs or related activities would seem implausible.

32
33 In the case of an accidental spill, the upper bound HQ for sensitive species of fish is 4. As
34 summarized at the top of Worksheet G03, this HQ is associated with a concentration of about 4.1
35 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
36 this relationship, the accidental spill modeled for the current risk assessment (Section 3.2.3.4.1)
37 would be expected to cause detectable and perhaps substantial levels of mortality in sensitive
38 species of fish. Whether or not an actual spill would cause fish mortality depends on the amount
39 of spinosad released into the water and the characteristics of the waterbody, including size and
40 water turnover or flow rates, and the sensitivities of the fish populations in the affected area.

41
42 As discussed in the following section, adverse effects on at least some aquatic invertebrates are
43 likely. Consistent with the conclusions in a previous EPA risk assessment (U.S.

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

3 **4.4.3.2. Amphibians (Aquatic Phase)**

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

8 **4.4.3.4. Aquatic Invertebrates**

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

13
14 In terms of peak/acute expected concentrations in water, the HQs bracket the level of concern.
15 For sensitive species, the HQs are 3 (0.04 to 77) for a single application and 6 (0.05 to 153) for
16 two applications. For tolerant species, the HQs are 0.07 (0.0008 to 1.7) for a single application
17 and 0.1 (0.001 to 3) for two applications. These broad ranges of HQs reflect the wide-range of
18 conditions (i.e., temperature, rainfall, and soil textures) used in the GLEAMS-Driver modelling
19 on which the exposure assessments are based (Section 3.2.3.4.3). Qualitatively, the HQs suggest
20 that it is unlikely that tolerant species of invertebrates would be adversely affected. For sensitive
21 species, however, the risk characterization is indefinite. In areas with a low potential for water
22 contamination, no adverse effects on even sensitive species of aquatic invertebrates might be
23 observed. In areas with a higher potential for water contamination, adverse effects and probably
24 substantial mortality would be noted in sensitive species of aquatic invertebrates.

25
26 In terms of longer-term risks to aquatic invertebrates, the risk characterization for sensitive
27 species is similar to that for acute exposures. The longer-term HQs are 1.1 (0.007 to 30) for a
28 single application and 2 (0.01 to 59) for two applications. For tolerant species, the chronic HQs
29 are below the level of concern even at the upper bounds of exposure. The highest HQ for
30 tolerant species is 0.3—i.e., the upper bound of the HQ tolerant species following two
31 applications.

32
33 The high variability in the estimated concentrations of spinosad in water precludes a general risk
34 characterization. In any site-specific application of spinosad, the risks will vary substantially
35 with local conditions. Further guidance on the variability in the concentrations of spinosad in
36 water can be gleaned from Appendix 8 (one application) and Appendix 9 (two applications). For
37 example, Table A8-7 gives the expected water contamination rates following a single
38 application. Relatively arid areas, particularly those with predominantly loam or sandy soil
39 textures, have the lowest water contamination rates. Much higher water contamination rates are
40 evident in areas with moderate or substantial rainfall. The specific average annual rainfalls for
41 the nine locations used in the modeling are listed in Table 10. Given the highly variable results
42 from the water modeling used in the current risk assessment and the substantial impact that this
43 variability has on the risk characterization for aquatic invertebrates, site-specific efforts to
44 estimate concentrations of spinosad in surface water might be justified, particularly in areas with
45 moderate to heavy rainfall.

1
2 In the case of an accidental spill, the risk characterization is simple and unequivocal. As
3 summarized at the top of Worksheet G03, the accidental spill scenarios estimate concentrations
4 of spinosad in water of about 1 (0.1 to 4) mg a.i./L. As summarized in Table 25 and discussed in
5 Section 4.1.3.3.1, most LC₅₀ values for spinosad formulations in aquatic invertebrates range from
6 0.0018 mg a.i./L to about 0.09 mg a.i./L. In the event of an accidental spill, the likelihood of
7 extensive mortality in many species of aquatic invertebrates is virtually certain. Substantial
8 mortality after an accidental spill is likely in even the most tolerant species—i.e., mosquitoes
9 with EC₅₀ values in the range of 0.2 to about 0.5 mg a.i./L. As is true in the accidental spill
10 scenario for fish (Section 4.4.3.1), the extent of mortality among aquatic invertebrates following
11 an actual spill would depend on the amount of spinosad released into the water and the
12 characteristics of the waterbody, including size and water turnover or flow rates.

13 ***4.4.3.4. Aquatic Plants***

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

22
23 In the event of an accidental spill, the HQs for sensitive species of algae—i.e., 20 (2 to 82)—
24 clearly indicate the potential for adverse effects. This potential is similar to (albeit less extreme
25 than) the anticipated effects on aquatic invertebrates following an accidental spill
26 (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.

DER01 DERs taken from U.S. EPA/OPP/HED 1997a or
http://iaspub.epa.gov/apex/pesticides/f?p=CHEMICALSEARCH:3:0::NO:1,3,31,7,12,25:P3_XCHEMICAL_ID:3922.

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.

{Abdollahi et al. 2004} Abdollahi M; Ranjbar A; Shadnia S; Nikfar S; Rezaie A. 2004. Pesticides and oxidative stress: a review. *Medical Science Monitor*. 10(6): RA141-147. [Std]

{Achaleke et al. 2009} Achaleke J; Martin T; Ghogomu RT; Vaissayre M; Brevault T. 2009. Esterase-Mediated Resistance to Pyrethroids in Field Populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Central Africa. *Pest Management Science*. 65(10): 1147-1154. [Set05]

{Aciole et al. 2014} Aciole EH; Guimarães NN; Silva AS; Amorim EM; Nunomura SM; Garcia AC; Cunha KS; Rohde C. 2014. Genetic Toxicity of Dillapiol and Spinosad Larvicides in Somatic Cells of *Drosophila melanogaster*. *Pest Management Science*. 70(4):559-65. [Set01]

{Agrawal and Sharma 2010} Agrawal A; Sharma B. 2010. Pesticides induced oxidative stress in mammalian systems. *International Journal of Biological and Medical Research*. 1(2): 90-104. [Std]

{Akmoutsou et al. 2011} Akmoutsou P; Mademtzoglou D; Nakou I; Onoufriadis A; Papadopoulou X; Kounatidis I; Frantzios G; Papadakis G; Vasiliadis K; Papadopoulos NT; Mavragani-Tsipidou P. 2011. Evaluation of Toxicity and Genotoxic Effects of Spinosad and Deltamethrin in *Drosophila melanogaster* and *Bactrocera oleae*. *Pest Management Science*. 67(12):1534-40. [Set01]

{Albee et al. 1994} Albee RR; Berdaxco NM; Yano BL. 1994. Data Evaluation Record (DER) for unpublished study: XDE-105: Acute neurotoxicity study in Fischer 344 rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43557501. 10 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Alix and Vergnet 2007} Alix A; Vergnet C. 2007. Risk assessment to honey bees: a scheme developed in France for non-sprayed systemic compounds. *Pest Management Science*. 63: 1069-1080. [Std]

{Allen and Fryrear 1997} Allen RR; Fryrear DW. 1977. Limited tillage saves soil, water, and energy. ASAE Annual Meeting, NC State Univ., Raleigh, NC. June 26-29, 1977. 14 pp. [Std]

{Amarasekare and Edelson 2004} Amarasekare KG; Edelson JV. 2004. Effect of temperature on efficacy of insecticides to differential grasshopper (Orthoptera: Acrididae). *Journal of Economic Entomology*. 97(5): 1595-1602. [Set00]

{Ambudkar et al. 2003} Ambudkar SV; Kimchi-Sarfaty C; Sauna ZE; Cottesman MM. 2003. P-glycoprotein: from genomics to mechanism. *Oncogene*. 22: 7468-7485. [Set00]

- {Anogwih et al. 2013} Anogwih JA; Makanjuola WA; Chukwu LO. 2013. Spinosad Induced Cytogenotoxic Effects on the Mosquito Fish, *Poecilia reticulata*. *Journal of Clinical Toxicology* S12:001. doi: 10.4172/2161-0495.S12-001. [Set00]
- {Antonio et al. 2008} Antonio G; Sanchez D; Williams T; Marina CF. 2008. Paradoxical effects of sublethal exposure to the naturally derived insecticide spinosad in the dengue vector mosquito, *Aedes aegypti*. Published online in Wiley Interscience: www.interscience.wiley.com. [Set00]
- {Alston and Tebeau 2011} Alston DF; Tebeau A. 2011. European Earwig (*Forficula auricularia*). Utah Pests Fact Sheet. ENT-145-11. Dated August 2011. Available at: <http://extension.usu.edu/files/publications/factsheet/earwig-orn.pdf>. [Set00]
- {Appleton 2008} Appleton H. 2008. Environmental risk and pest management issues at the Worcester ALB site associated with the use of injected imidacloprid (Merit) chemical control. Unpublished analysis dated December 4, 2008. [Set00]
- {Awkerman et al. 2008} Awkerman JA; Raimondo S; Barron MG. 2008. Development of species sensitivity distributions for wildlife using interspecies toxicity correlation models. *Environmental Science and Technology*. 42(9):3447-52. [Std]
- {Bailey et al. 2005} Bailey J; Scott-Dupree C; Harris R; Tolman J; Harris B. 2005. Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. *Apidologie*. 36(4): 623-633. [Set00]
- {Bao et al. 2014} Bao WX; Narai Y; Nakano A; Kaneda T; Murai T; Sonoda S. 2014. Spinosad Resistance of Melon Thrips, *Thrips palmi*, is Conferred by G275e Mutation in Alpha-Subunit of Nicotinic Acetylcholine Receptor and Cytochrome P450 Detoxification. *Pesticide Biochemistry and Physiology*. 112:51-5. [Set01]
- {Barbosa et al. 2015} Barbosa WF; Tome HV; Bernardes RC; Siqueira MA; Smaghe G; Guedes RN. 2015. Biopesticide-induced behavioral and morphological alterations in the stingless bee *Melipona quadrifasciata*. *Environmental Toxicology and Chemistry*. 34(9):2149-58. [Set06]
- {Bargańska et al. 2013} Bargańska Z; Ślebiodab M; Namieśnika J. 2013. Pesticide residues levels in honey from apiaries located of Northern Poland. *Food Control*. 31(1): 196-201. [Set04]
- {Baur et al. 2003} Baur ME; Ellis J; Hutchinson K; Boethel DJ. 2003. Contact Toxicity of Selective Insecticides for Non-Target Predaceous Hemipterans in Soybeans. *Journal of Entomological Science*. 38(2): 269-277. [Set01 ECOTOX-82481]
- {Beavers et al. 1994a} Beavers JB; Chafey K; Mitchell LR; Jaber M. 1994a. Data Evaluation Record (DER) for unpublished study: XDE-105 insecticide: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*). Wildlife International, Ltd., Lab. Report ID: 103-387, sponsored by DowElanco. MRID No. 43414533. 39 pp. [DER01]
- {Beavers et al. 1994b} Beavers JB; Chafey K; Mitchell LR; Jaber M. 1994b. Data Evaluation Record (DER) for unpublished study: XDE-105 insecticide: A Reproduction Study with the Mallard (*Anas platyrhynchos*). Wildlife International, Ltd., Lab. Report ID: 103-388, sponsored by DowElanco. MRID No. 43414532. 39 pp. [DER01]
- {Beloti et al. 2015} Beloti VH; Alves GR; Araujo DFD; et al. 2015. Lethal and Sublethal Effects of Insecticides Used on Citrus, on the Ectoparasitoid *Tamarixia radiata*. *PLOSone*. DOI: 10.1371/journal.pone.0132128. [Set00]
- {Benamu et al. 2007} Benamu MA; Schneider MI; Pineda S; Sanchez NE; Gonzalez A. 2007. Sublethal Effects of Two Neurotoxic Insecticides on *Araneus pratensis* (Araneae: Araneidae). *Communications in Agricultural and Applied Biological Sciences*. 72(3):557-9. [Set01 - Toxline01]
- {Benamu et al. 2013} Benamu MA; Schneider MI; Gonz lez A; Sanchez NE. 2013. Short and Long-Term Effects of Three Neurotoxic Insecticides on Biological and Behavioural Attributes of the Orb-Web Spider *Alpaida veniliae* (Araneae, Araneidae): Implications for IPM Programs. *Ecotoxicology*. 22(7):1155-64. [Set01 - Toxline01]

{Berard and Santonin 1996} Berard DF; Santonin DK. 1996. Characterization of Spinosad Residues in Cabbage from Single and Repeat Applications. Abstracts of Papers American Chemical Society. 211(1-2): Agro 40. [Set01]

{Besard et al. 2011} Besard L; Mommaerts V; Abdu-Alla G; Smagghe G. 2011. Lethal and Sublethal Side-Effect Assessment Supports a More Benign Profile of Spinetoram Compared with Spinosad in the Bumblebee *Bombus terrestris*. Pest Management Science. 67(5):541-7. [Set01]

{Beugnet et al. 2011} Beugnet F; Doyle V; Murray M; Chalvet-Monfray K. 2011. Comparative Efficacy on Dogs of a Single Topical Treatment with the Pioneer Fipronil/(s)-Methoprene and an Oral Treatment with Spinosad Against *Ctenocephalides felis*. Parasite. 18(4):325-31. [Set00 - Toxline01]

{Bielza et al. 2007} Bielza P; Quinto V; Contreras J; Torn, M; Mart;n A; Espinosa PJ. 2007. Resistance to Spinosad in the Western Flower Thrips, *Frankliniella occidentalis* (Pergande), in Greenhouses of South-Eastern Spain. Pest Management Science. 63(7):682-7. [Set01]

{Bielza et al. 2008} Bielza P; Quinto V; Gr valos C; Fern ndez E; Abell n J; Contreras J. 2008. Stability of Spinosad Resistance in *Frankliniella occidentalis* (Pergande) Under Laboratory Conditions. Bulletin of Entomological Research. 98(4):355-9. [Set01]

{Biondi et al. 2012} Biondi l A; Mommaerts V; Smagghe G; Viñuela E; Zappalà L; Desneux N. 2012. The non-target impact of spinosyns on beneficial arthropods. Pest Management Science. 68:1523–1536 [Set00]

{Biondi et al. 2013} Biondi A; Zappal... L; Stark JD; Desneux N. 2013. Do Biopesticides Affect the Demographic Traits of a Parasitoid Wasp and its Biocontrol Services Through Sublethal Effects?. PloS one. 8(9):e76548. [Set00]

{Boller et al. 2005} Boller EF; Vogt H; Terns P; Malavolta C. 2005. Working Document on Selectivity of Pesticides (2005). International Organisation for Biological and Integrated Control of Noxious Animals and Plants: West Palaearctic Regional Section. Available at: https://www.iobc-wprs.org/ip_ipm/03021_IOBC_WorkingDocumentPesticides_Explanations.pdf. [Std]

{Bond et al. 1995a} Bond D; Stebbins K; McGuirk R. 1995a. Data Evaluation Record (DER) for unpublished study: XDE-105: 18-Month dietary oncogenicity study in CD-1 mice. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43701505. 23 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Bond et al. 1995b} Bond DM; Yano BL; Stebbins KE. 1995b. . Data Evaluation Record (DER) for unpublished study: XDE-105: Two year chronic toxicity, neurotoxicity, and oncogenicity study in Fischer rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43701507 and 43710503. 22 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Boyd and Boethel 1998} Boyd ML; Boethel DJ. 1998. Susceptibility of Predaceous Hemipteran Species to Selected Insecticides on Soybean in Louisiana. Journal of Economic Entomology. 91 (2): 401-409. [Set01]

{Breslin et al. 1994} Breslin W; Quast J; Vedula U. 1994. Data Evaluation Record (DER) for unpublished study: XDE-105: Two generation dietary reproduction study in Sprague-Dawley rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43701506. 32 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Breslin et al. 2000} Breslin WJ; Marty MS; Vedula UV; Liberacki AB; Yano BL. 2000. Developmental Toxicity of Spinosad Administered by Gavage to Cd Rats and New Zealand White Rabbits. Food and Chemical Toxicology. 38(12):1103-12. [Set01]

{Brunner et al. 2001} Brunner JF; Dunley JE; Doerr MD; Beers EH. 2001. Effect of Pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), Parasitoids of Leafrollers in Washington. Journal of Economic Entomology. 94(5): 1075-1084. [EXOTOX-63713]

{Burns et al. 2001} Burns RE; Harris DL; Moreno DS; Eger IE. 2001. Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera:Tephritidae) in commercial citrus in Florida. *Florida Entomologist*. 84:672-678. [Set04]

{Cabrera-Marin et al. 2015} Cabrera-Marin NV; Liedo P; Vandame R; Sanchez D. 2015. Foraging Allocation in the Honey Bee, *Apis Mellifera* L. (Hymenoptera, Apidae), Tuned by the Presence of the Spinosad-Based Pesticide GF-120. *Neotropical Entomology*. 44(2):166-72. [Set01]

{Campos et al. 2014} Campos MR; Rodrigues AR; Silva WM; Silva TB; Silva VR; Guedes RN; Siqueira HA. 2014. Spinosad and the Tomato Borer *Tuta absoluta*: A Bioinsecticide, An Invasive Pest Threat, and High Insecticide Resistance. *PLoS one*. 9(8):e103235. [Set00]

{Carvalho et al. 2013} Carvalho SM; Belzunces LP; Carvalho GA; Brunet JL; Badiou-Beneteau A. 2013. Enzymatic Biomarkers as Tools to Assess Environmental Quality: A Case Study of Exposure of the Honeybee *Apis mellifera* to Insecticides. *Environmental Toxicology and Chemistry*. 32(9):2117-24. [Set01]

{CDPR 2002} CDPR (California Department of Pesticide Regulation). 2002. Facts about Spinosad. Report dated December 5, 2002. Available at: http://www.cdpr.ca.gov/docs/emon/epests/mexfly/fact_sheet.pdf.

{CDPR 2003} CDPR (California Department of Pesticide Regulation). No date. Environmental Fate of Spinosad. Prepared by Wynetta S. Kollman, Ph.D. Available at: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/spinosad_fate.pdf.

{CDPR 2003} CDPR (California Department of Pesticide Regulation). No date. Environmental Fate of Spinosad. Prepared by Wynetta S. Kollman, Ph.D. Available at: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/spinosad_fate.pdf.

{CDPR 2015} CDPR (California Department of Pesticide Regulation). 2015. Summary of Pesticide Use Report Data 2013, Indexed by Chemical. Report dated May 2015. Available at: www.cdpr.ca.gov/docs/pur/pur13rep/13sum.htm. [Std]

{Cetin et al. 2005} Cetin H; Yanikoglu A; Cilek JE. 2005. Evaluation of the Naturally-Derived Insecticide Spinosad Against *Culex pipiens* L. (Diptera: Culicidae) Larvae in Septic Tank Water in Antalya, Turkey. *Journal of Vector Ecology*. 30(1):151-4. [Set01]

{ChemIDplus 2015a} ChemIDplus. 2015a. United States National Library of Medicine. Entry for spinosad. Available at: <http://chem.sis.nlm.nih.gov/chemidplus/rn/startswith/168316-95-8>. [Std]

{ChemIDplus 2015b} ChemIDplus. 2015b. United States National Library of Medicine. Entry for Spinosyn A. Available at: <http://chem.sis.nlm.nih.gov/chemidplus/rn/startswith/131929-60-7>. [Std]

{ChemIDplus 2015c} ChemIDplus. 2015c. United States National Library of Medicine. Entry for Spinosyn D. Available at: <http://chem.sis.nlm.nih.gov/chemidplus/rn/startswith/131929-60-7>. [Std]

{Cisneros et al. 2002} Cisneros J; Goulson D; Derwent LC; Penagos DI; Hernández O; Williams T. 2002. Toxic effects of Spinosad on predatory insects. *Biological Control*. 23: 156–163. [Set00]

{Clarke 2011} Clarke. 2011. Natular®, Naturally Derived Active Ingredient. Larvicide for Mosquito Control. Brochure dated 2011 and available at: <http://www.solcomercio.com.br/controle/uploads/bulas/158.pdf> [Set06].

{Cleveland et al. 2002a} Cleveland CB; Bormett GA; Saunders DG; Powers FL; McGibbon AS; Reeves GL; Rutherford L; Balcer JL. 2002a. Environmental Fate of Spinosad. 1. Dissipation and Degradation in Aqueous Systems. *Journal of Agricultural and Food Chemistry*. 50(11):3244-56. [Set01]

{Cleveland et al. 2002b} Cleveland CB; Mayes MA; Cryer SA. 2002b. An Ecological Risk Assessment for Spinosad Use on Cotton. *Pest Management Science*. 58(1):70-84. [Set01]

- {Clydesdale 1997} Clydesdale, FM. 1997. Food Additives: Toxicology, Regulation, and Properties. CRC Press, Boca Raton, Florida. CD-ROM Database.[Std]
- {Cole and Lundquist 2011} Cole SQ; Lundquist LM. 2011. Spinosad for Treatment of Head Lice Infestation. The Annals of Pharmacotherapy. 45: 954-959. [Set00]
- {Coon 2005} Coon MJ. 2005. Cytochrome P450: Nature's Most Versatile Biological Catalyst. Annual Review of Pharmacology and Toxicology. 45: 1-25. [Std]
- {Cranshaw et al. 2014} Cranshaw W; Aguayo I; Leatherman D. Douglas-fir Tussock Moths, Fact Sheet No. 5.542. Insect Series, Trees and Shrubs. Document revised July, 2014. Available at: <http://extension.colostate.edu/docs/pubs/insect/05542.pdf>. [Set00]
- {Darriet et al. 2005} Darriet F; Duchon S; Hougard JM. 2005. Spinosad: A New Larvicide Against Insecticide-Resistant Mosquito Larvae. Journal of the American Mosquito Control Association. 21(4):495-6. [Set00]
- {Davies and Morris 1993} Davies B; Morris T. 1993. Physiological Parameters in Laboratory Animals and Humans. Pharmaceutical Research. 10(7): 1093-1095. [Std-PBPK]
- {de Freitas Bueno et al. 2008} de Freitas Bueno A; de Freitas Bueno RCO; Parra JRP; Vieira SS. 2008. Effects of Pesticides Used in Soybean Crops to the Egg Parasitoid *Trichogramma pretiosum*. Ciência Rural. 38(6): 1495-1503. [Set00]
- {Deardorff and Stark 2009} Deardorff AD; Stark JD. 2009. Acute Toxicity and Hazard Assessment of Spinosad and R-11 to Three Cladoceran Species and Coho Salmon. Bulletin of Environmental Contamination and Toxicology. 82(5):549-53. [Set01]
- {Deardorff and Stark 2011} Deardorff AD; Stark JD. 2011. Population-Level Toxicity of the Insecticide, Spinosad and the Nonylphenol Polyethoxylate, R-11, to the Cladoceran Species *Ceriodaphnia dubia* Richard. Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes. 46(4):336-40. [Set01]
- {Dively and Kamel 2012} Dively GP; Kamel A. 2012. Insecticide Residues in Pollen and Nectar of a Cucurbit Crop and Their Potential Exposure to Pollinators. Journal of Agricultural and Food Chemistry. 60(18):4449-56. [Set01]
- {Domoradzki and Shabrang 1996} Domoradzki JY; Shabrang SN. 1996. Spinosyn A: Probe study dermal absorption of ¹⁴C-labelled spinosyn A in Fischer rats. Unpublished report No. DR-0323-1194-029. Submitted to WHO by Dow AgroSciences, Letcombe, United Kingdom. As summarized in FAO/WHO 2001. [Sec]
- {Dow 2014a} Dow (The Dow Chemical Company). 2014a. Spinosad: Product Safety Assessment. Revised October 31, 2014. Available at: http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_091c/0901b8038091c93b.pdf?filepath=productsafety/pdfs/noreg/233-00381.pdf&fromPage=GetDoc. [Set00]
- {Dow 2014b} Dow (The Dow Chemical Company). 2014b. Spinetoram: Product Safety Assessment. Revised October 24, 2014. Available at: http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_091b/0901b8038091bc16.pdf?filepath=productsafety/pdfs/noreg/233-00382.pdf&fromPage=GetDoc [Set00]
- {DowElanco 1996} DowElanco (Now Dow AgroSciences). 1996. Spinosad technical guide. 25 pp. Available at: <http://legacy-dc.ucsf.edu/tid/gr185c00;jsessionid=934FF90E13CAC3085FAB302276DA08EB.tobacco03>. [Set00]
- {Duchet et al. 2008} Duchet C; Larroque M; Caquet T; Franquet E; Lagneau C; Lagadic L. 2008. Effects of Spinosad and *Bacillus thuringiensis israelensis* on a Natural Population of *Daphnia pulex* in Field Microcosms. Chemosphere. 74(1):70-7. [Set01]

{Duchet et al. 2010a} Duchet C; Caquet T; Franquet E; Lagneau C; Lagadic L. 2010a. Influence of Environmental Factors on the Response of a Natural Population of *Daphnia magna* (Crustacea: Cladocera) to Spinosad and *Bacillus thuringiensis israelensis* in Mediterranean Coastal Wetlands. *Environmental Pollution*. 158(5):1825-33. [Set01]

{Duchet et al. 2010b} Duchet C; Coutellec MA; Franquet E; Lagneau C; Lagadic L. 2010b. Population-Level Effects of Spinosad and *Bacillus thuringiensis israelensis* in *Daphnia pulex* and *Daphnia magna*: Comparison of Laboratory and Field Microcosm Exposure Conditions. *Ecotoxicology*. 19(7):1224-37. [Set01]

{Duchet et al. 2011} Duchet C; Mitie Inafuku M; Caquet T; Larroque M; Franquet E; Lagneau C; Lagadic L. 2011. Chitobiase Activity as An Indicator of Altered Survival, Growth and Reproduction in *Daphnia pulex* and *Daphnia magna* (Crustacea: Cladocera) Exposed to Spinosad and Diflubenzuron. *Ecotoxicology and Environmental Safety*. 74(4):800-10. [Set01]

{Duchet et al. 2015} Duchet C; Franquet E; Lagadic L; Lagneau C. 2015. Effects of *Bacillus thuringiensis israelensis* and Spinosad on Adult Emergence of the Non-Biting Midges *Polypedilum nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in Coastal Wetlands. *Ecotoxicology and Environmental Safety*. 115:272-8. [Set01]

{Dunn et al. 2011} Dunn ST; Hedges L; Sampson KE; Lai Y; Mahabir S; Balogh L; Locuson CW. 2011. Pharmacokinetic Interaction of the Antiparasitic Agents Ivermectin and Spinosad in Dogs. *Drug Metabolism and Disposition*. 39(5):789-95. [Set01]

{Dunning. 1993} Dunning JB. 1993. *CRC Handbook of Avian Body Masses*. CRC Press, Boca Raton FL, 371 pp.[Std]

{Durkin et al. 1995} Durkin PR; Rubin L; Withey J; Meylan W. 1995. Methods of assessing dermal absorption with emphasis on uptake from contaminated vegetation. *Toxicology and Industrial Health*. 11(1): 63-79.[Std]

{Ecobichon. 1998} Ecobichon DJ. 1998. *Occupational Hazards of Pesticide Exposure – Sampling, Monitoring, Measuring*. Taylor & Francis, Philadelphia, PA. 251 pp.[Std]

{ECOTOX 2016} ECOTOX. 2016. ECOTOX Release 4.0. The ECOTOX (ECOTOXicology) Database. Terrestrial report on spinosad. Available at: <http://cfpub.epa.gov/ecotox/>. [Std]

{Edwards and Bohlen 1992} Edwards CA; Bohlen PJ. 1992. The Effects of Toxic Chemicals on Earthworms. *Reviews of Environmental Contamination and Toxicology*. 125: 23-99. [Std-Compendia]

{Eelen et al. 2006} Eelen H; Gobin B; Miles M. 2006. Field Studies to Determine the Effects of Spinosad on the Predatory Bugs *Anthocoris nemoralis* and *A. Nemorum*. *Communications in Agricultural and Applied Biological Sciences*. 71(2 Pt B):429-32. [Set01]

{EFSA 2011} EFSA (European Food Safety Authority). 2011. Reasoned Opinion: Modification of the existing MRLs for spinosad in various crops. *EFSA Journal* 2011;9(9):2352. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2352.pdf. [Set00]

{EFSA 2012} EFSA (European Food Safety Authority). 2012. Statement of EFSA: Statement on the modification of the existing MRL for spinosad in raspberries. *EFSA Journal* 2012;10(5):2751. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2751.pdf. [Set00]

{EFSA 2013} EFSA (European Food Safety Authority). 2013. Reasoned opinion on the modification of the existing MRLs for spinosad in small fruit and berries and several commodities of animal origin1. *EFSA Journal* 2013;11(11):3447. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3447.pdf. [Set00]

{EFSA 2014} EFSA (European Food Safety Authority). 2014. Scientific Report of EFSA. The 2012 European Union Report on pesticide residues in food. *EFSA Journal* 2014;12(12):3942. Available at: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3942.pdf. [Set00]

- {Elanco 2012} Elanco. 2012. Elanco Companion Animal Health Technical Bulletin: Comfortis® (spinosad) Feline Safety and Efficacy Overview. Available at: <https://www.elancovet.com/pdf/safety-and-efficacy-white-paper-cat.pdf>. [Set00]
- {Elanco Animal Health 2007} Elanco Animal Health. 2007. Corrected Freedom of Information Summary: Original New Animal Drug Application. NADA 141-277. Comfortis: Spinosad chewable tablets: Dogs. Sponsored by Elanco Animal Health, A Division of Eli Lilly & Co. Date of Approval: September 25, 2007. 29 pp. [DER01]
- {El-Hoda et al. 2012} El-Hoda N; Zidan A; Galal OA. 2012. Evaluation of Hematological, Cytogenetical and Biochemical Effects of Malathion and Spinosad on Male Albino Rats. *Amer-Eurasian J Toxicol Sci.* 4(2): 118-130. [Set00]
- {Elskus 2007} Elskus AA. 2007. Pilot Study of Sublethal Effects on Fish of Pesticides Currently Used and Proposed for Use on Maine Blueberries. U. S. Geological Survey Open File Report 2007-1110, U. S. Geological Survey, Reston, VA. 14 p. Available at: http://pubs.usgs.gov/of/2007/1110/pdf/OFR_2007-1110.pdf. [Set01 ECOTOX]
- {Elzen and Elzen 1999} Elzen GW; Elzen PJ. 1999. Lethal and Sublethal Effects of Selected Insecticides on *Geocoris punctipes*. *Southwestern Entomologist.* 24(3): 199-205. [Set01 ECOTOX-63850]
- {Elzen et al. 1999} Elzen GW; Rojas MG; Elzen PJ; King EG; Barcenas NM. 1999. Toxicological Responses of the Boll Weevil (Coleoptera: Curculionidae) Ectoparasitoid *Catolaccus grandis* (Hymenoptera: Pteromalidae) to Selected Insecticides. *Journal of Economic Entomology.* 92 (2): 309-313. [Set01/ECOTOX-63851]
- {European Commission 2006} European Commission. 2006. Review report for the active substance spinosad. Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 14 July 2006 in view of the inclusion of spinosad in Annex I of Directive 91/414/EEC. 45 pp. [Set00]
- {Fan et al. 2008} Fan S; Segawa R; Kim D. et al. 2008. Environmental Monitoring Results of Spinosad Aerial Applications for the Mexican Fruit Fly Eradication In Valley Center, San Diego County, 2003. California Department of Pesticide Regulation. Available at: http://www.cdpr.ca.gov/docs/emon/epests/mexfly/mxff_final.pdf. [Set00]
- {FAO/WHO 2001} FAO/WHO. 2001. Spinosad. Pesticide residues in food. Toxicological evaluations: Spinosad. Available at: <http://www.inchem.org/documents/jmpr/jmpmono/2001pr12.htm>. [Set00]
- {Fletcher et al. 1994} Fletcher JS; Nellessen JE; Pflieger TG. 1994. Literature review and evaluation of the EPA food-chain (Kenega) nomogram, an instrument for estimating pesticide residues on plants. *Environ. Toxicol. Chem.* 13(9):1383-1391. [Std]
- {Franc and Bouhsira 2009} Franc M; Bouhsira E. 2009. Evaluation of Speed and Duration of Efficacy of Spinosad Tablets for Treatment and Control of *Ctenocephalides canis* (Siphonaptera: Pulicidae) Infestations in Dogs. *Parasite.* 16(2):125-8. [Set00]
- {Franklin et al. 2004} Franklin MT; Winston ML; Morandin LA. 2004. Effects of Clothianidin on *Bombus impatiens* (Hymenoptera: Apidae) Colony Health and Foraging Ability. *Ecotoxicology.* 97(2): 369-373. [Imid]
- {Gao et al. 2007a} Gao JR; Deacutis JM; Scott JG. 2007a. The nicotinic acetylcholine receptor subunits M α 5 and M δ 3 on autosome 1 of *Musca domestica* are not involved in spinosad resistance. *Insect Molecular Biology.* 16(6):691-701. [Set00]
- {Gao et al. 2007b} Gao R; Dong J; Zhang W; Chen WL. 2007b. Dietary Risk Assessment of Spinosad in China. *Regulatory Toxicology and Pharmacology.* 49(1):31-42. [Set01]
- {George et al. 2010} George DR; Shiel RS; Appleby WG; Knox A; Guy JH. 2010. *In vitro* and *in vivo* Acaricidal Activity and Residual Toxicity of Spinosad to the Poultry Red Mite, *Dermanyssus gallinae*. *Veterinary Parasitology.* 173(3-4):307-16. [Set01]

{Gilbert et al. 1994} Gilbert KS; Johnson KA; Stebbins KA. 1994. Data Evaluation Record (DER) for unpublished study: XDE-105: Acute oral toxicity study in Fischer 344 rats and CD-1 mice. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43414515. 4 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Gilliom et al. 2007} Gilliom RJ; Barbash JE; Crawford CG; et al. 2007. Pesticides in the Nation's Streams and Ground Water, 1992–2001. USGS National Water-Quality Assessment Program Circular 1291. Revised February 15, 2007. Available at: <http://pubs.usgs.gov/circ/2005/1291/>. [Std]

{Goldstein et al. 1974} Goldstein A; Aronow L; Kaman SM. 1974. Principles of Drug Action: The Basis of Pharmacology. 2nd ed. John Wiley and Sons, New York, NY. 854 p.[Std]

{Gomez-Escobar et al. 2014} Gomez-Escobar E; Liedo P; Montoya P; Vandame R; Sanchez D. 2014. Behavioral Response of Two Species of Stingless Bees and the Honey Bee (Hymenoptera: Apidae) to Gf-120. Journal of Economic Entomology. 107(4):1447-9. [Set00 - Toxline01]

{Greenbook 2015} Greenbook. 2015. Forestry Formulations of Spinosad. Available at: www.greenbook.net. Downloaded on November 20, 2015. [Set00]

{Guedes 2016} Guedes RN. 2016. Response to Minor Query on "Biopesticide-induced behavioral and morphological alterations...". Email from Raul Guedes (Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil) to Patrick Durkin (SERA Inc.) dated September 7, 2016. [Set06]

{Gunning et al. 2012} Gunning K; Pippitt K; Kiraly B; Sayler M. 2012. Pediculosis and Scabies: Treatment Update. Am Fam Physician. 86(6):535-41. [Set00]

{Haile et al. 1999} Haile FJ; Peterson R KD; Higley LG. 1999. Gas-Exchange Responses of Alfalfa and Soybean Treated with Insecticides. Journal of Economic Entomology. 92 (4): 954-959. [Set01/ECOTOX-64569]

{Hall and Barry 1995} Hall FR; Barry JW. 1995. An Overview of Biorational Pest Control Agents. Chapter 1 in: Biorational Pest Control Agents, Formulation and Delivery. ACS Symposium Series 595. pp. 1-18. Available at: <http://pubs.acs.org/isbn/9780841232266>. [Set00]

{Halm et al. 2006} Halm MP; Rortais A; Arnold G; Tasi JN; Rault S. 2006. New Risk Assessment Approach for Systemic Insecticides: The Case of Honey Bees and Imidacloprid (Gaucho). Environmental Science and Technology. 40(7):2448-54. [Set01]

{Hanley et al. 2002} Hanley T Jr; Breslin WJ; Quast JF; Carney EW. 2002. Evaluation of Spinosad in a Two-Generation Dietary Reproduction Study Using Sprague-Dawley Rats. Toxicological Sciences. 67(1):144-52. [Set01]

{Hanley et al. 2002} Hanley TR Jr; Breslin WJ; Quast JF; Carney EW. 2002. Evaluation of spinosad in a two-generation dietary reproduction study using Sprague-Dawley rats. Toxicological Sciences. 64: 144-152. [Set00]

{Harada 1995} Harada T. 1995. . Data Evaluation Record (DER) for unpublished study: XDE-105: 12-Month oral chronic toxicity study in dogs. The Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodairashi, Tokyo 187, Japan. Lab Proj. Study ID IET 91-0080. MRID No. 43701504. 15 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Harrell and Stepanek 2005} Harrell M; Stepanek L. 2005. Chemical Control of Common Forest and Shade Tree Pests. Nebraska Forest Service / University of Nebraska Lincoln. Available at: <http://nrc1.nrc.state.ne.us/epubs/u2140/h004-2005.pdf>. [Set00]

{Henics and Wheatley 1999} Henics T; Wheatley DN. 1999. Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features. Biology of the Cell. 91(7):485-98. [Set04]

{Herzog et al. 2002} Herzog GA; McPherson RM; Jones DC; Ottens RJ. 2002. Baseline Susceptibility of Tobacco Hornworms (Lepidoptera: Sphingidae) to Acephate, Methomyl and Spinosad in Georgia. *Journal of Entomological Science*. 37(1): 94-100. [Set05]

{Hoerger and Kenaga. 1972} Hoerger F; Kenaga EE. 1972. Pesticide residues on plants: Correlation of representative data as a basis for estimation of their magnitude in the environment. In: *Environmental Quality and Safety, Volume I: Global Aspects of Toxicology and Technology as Applied to the Environment*. F. Coulston and F. Kerte (eds.). Academic Press, New York, NY. pp. 9-28.[Std]

{Holt et al. 2006} Holt KM; Opit GP; Nechols JR; Margolies DC. 2006. Testing for Non-Target Effects of Spinosad on Twospotted Spider Mites and Their Predator *Phytoseiulus persimilis* Under Greenhouse Conditions. *Experimental and Applied Acarology*. 38(2-3):141-9. [Set01]

{Horowitz et al. 2009} Horowitz AR; Ellsworth PC; Ishaaya I. 2009. Biorational Pest Control: An Overview. In: *Biorational Control of Arthropod Pests*. pp. 1-20. Edited by: Ishaaya, Isaac, Horowitz, A. Rami. Published by Springer. ISBN: 978-90-481-2316-2. Available at: http://link.springer.com/chapter/10.1007/978-90-481-2316-2_1. [Set01]

{Hou et al. 2014} Hou W; Liu Q; Tian L; Wu Q; Zhang Y; Xie W; Wang S; Miguel KS; Funderburk J; Scott JG. 2014. The α_6 Nicotinic Acetylcholine Receptor Subunit of *Frankliniella Occidentalis* Is Not Involved in Resistance to Spinosad. *Pesticide Biochemistry and Physiology*. 111:60-7. [Set01]

{Hoxter et al. 1992} Hoxter KA; Bernard WL; Beavers JB. 1992. Data Evaluation Record (DER) for unpublished study: XDE-105: An acute contact toxicity with the honey bee. Wildlife International, Ltd., Easton, MD. Lab. Report No. 103-384. MRID 43414547. [DER01]

{HSDB 2003} HSDB (Hazardous Substances Database). Record for Spinosyn A. Last updated September 12, 2003. <http://toxnet.nlm.nih.gov>. [Set00]

{HSDB 2015} HSDB (Hazardous Substances Database). Record for Propylene Glycol. Last updated October 15, 2015. <http://toxnet.nlm.nih.gov>. [Set00]

{Hsu and Feng 2006} Hsu JC; Feng HT. 2006. Development of Resistance to Spinosad in Oriental Fruit Fly (Diptera: Tephritidae) in Laboratory Selection and Cross-Resistance. *Journal of Economic Entomology*. 99(3):931-6. [Set01]

{Hsu et al. 2012a} Hsu JC; Haymer DS; Chou MY; Feng HT; Chen HH; Huang YB; Mau RF. 2012a. Monitoring Resistance to Spinosad in the Melon Fly (*Bactrocera cucurbitae*) in Hawaii and Taiwan. *Scientific World Journal*. Article ID 750576, 8 pages. doi:10.1100/2012/750576. [Set00]

{Hsu et al. 2012b} Hsu JC; Feng HT; Wu WJ; Geib SM; Mao CH; Vontas J. 2012b. Truncated Transcripts of Nicotinic Acetylcholine Subunit Gene *Bd α_6* Are Associated with Spinosad Resistance in *Bactrocera dorsalis*. *Insect Biochemistry and Molecular Biology*. 42(10):806-15. [Set00]

{Huang et al. 2004} Huang F; Subramanyam B; Toews MD. 2004. Susceptibility of Laboratory and Field Strains of Four Stored-Product Insect Species to Spinosad. *Journal of Economic Entomology*. 97(6):2154-9. [Set01]

{Humphrey and Dykes 2008} Humphrey JAC; Dykes ES. 2008. Thermal energy conduction in a honey bee comb due to cell-heating bees. *J Theoretical Biology*. 250 (1): 194-208. [Std]

{Hussain et al. 2009} Hussain R; Ashfaq M; Saleem MA. 2009. Biochemical abnormalities produced by spinosad in *Tribolium castaneum* Adult Beetles. *Inter. J. Agri. Biol*. 11: 241-244. [Set00]

{Infante-Rodríguez et al. 2011} Infante-Rodríguez DA; Novelo-Gutiérrez R; Mercado G; Williams T. 2011. Spinosad Toxicity to *Simulium* spp. Larvae and Associated Aquatic Biota in a Coffee-Growing Region of Veracruz State, Mexico. *Journal of Medical Entomology*. 48(3):570-6. [Set01]

{IRAC 2016} IRAC (Insecticide Resistance Action Committee). 2016. IRAC Mode of Action Classification Scheme. Prepared by: IRAC International MoA Working Group, Approved by: IRAC Executive. Version 8.1, Issued April 2016. Available at: <http://www.irac-online.org/documents/moa-classification/>. [Std]

{Jiang and Mulla 2009} Jiang Y; Mulla MS. 2009. Laboratory and Field Evaluation of Spinosad, a Biorational Natural Product, Against Larvae of *Culex* Mosquitoes. *Journal of the American Mosquito Control Association*. 25(4):456-66. [Set01]

{Jirk et al. 1995} Jirk HD; Landre AM; Miller JA; Hugo JM; Martin MD. 1995. Data Evaluation Record (DER) for unpublished study: XDE-105: Evaluation of the chronic toxicity of XDE-105 insecticide to the daphnid, *Daphnia magna* straus following flow-through exposure. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43848801. 16 pp. [DER01]

{Jones and Ottea 2013} Jones OM; Ottea J. 2013. The Effects of Spinosad on *Culex quinquefasciatus* and Three Nontarget Insect Species. *Journal of the American Mosquito Control Association*. 29(4):346-51. [Set01]

{Jones et al. 2005} Jones T; Scott-Dupree C; Harris R; Shipp L; Harris B. 2005. The Efficacy of Spinosad Against the Western Flower Thrips, *Frankliniella occidentalis*, and its Impact on Associated Biological Control Agents on Greenhouse Cucumbers in Southern Ontario. *Pest Management Science*. 61(2):179-85. [Set01]

{Kano and Rubin 2010} Kano S; Rubin BK. 2010. Mechanisms of Action and Clinical Application of Macrolides as Immunomodulatory Medications. *Clinical Microbiology Reviews*. 23(3): 590–615. [Set00]

{Kegley et al. 2014} Kegley SE; Hill BR; Orme S; Choi AH. Spinosad: PAN Pesticide Database, Pesticide Action Network, North America (Oakland, CA, 2014), <http://www.pesticideinfo.org>. © 2000-2014 Pesticide Action Network, North America. All rights reserved. [Set00]

{Khan and Akram 2014} Khan HA; Akram W. 2014. The Effect of Temperature on the Toxicity of Insecticides Against *Musca domestica* L.: Implications for the Effective Management of Diarrhea. *PloS one*. 9(4):e95636. [Set00]

{Khan et al. 2011a} Khan HA; Akram W; Shehzad K; Shaalan EA. 2011. First Report of Field Evolved Resistance to Agrochemicals in Dengue Mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. *Parasit Vectors*. 4:146. [Set01 ECOTOX-167183]

{Khan et al. 2011b} Khan M; Hossain MA; Khan SA; Islam MS; Chang CL. 2011b. Development of Liquid Larval Diet With Modified Rearing System For *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) for the Application of Sterile Insect Technique. *ARNP Journal of Agricultural and Biological Science*. 6(10): 52-57. [Set00]

{Kirst et al. 1992} Kirst HA; Michel KJ; Mynderse JS; et al. 1992. Discovery, Isolation, and Structure Elucidation of a Family of Structurally Unique, Fermentation-Derived Tetracyclic Macrolides. Chapter 20 in *Synthesis and Chemistry of Agrochemicals III*. ACS Symposium Series. 504: 214–225. Available at: <http://pubs.acs.org/doi/abs/10.1021/bk-1992-0504.ch020>. [Set01]

{Knisel and Davis 2000} Knisel WG; Davis FM. 2000. GLEAMS (Groundwater Loading Effects of Agricultural Management Systems), Version 3.0, User Manual. U.S. Department of Agriculture, Agricultural Research Service, Southeast Watershed Research Laboratory, Tifton, GA. Pub. No.: SEWRL-WGK/FMD-050199. Report Dated May 1, 1999 and revised August 15, 2000. 194pp.[Std]

{Kovac et al. 2010} Kovac H; Stabentheiner A; Schmaranzer S. 2010. Thermoregulation of water foraging honeybees—Balancing of endothermic activity with radiative heat gain and functional requirements. *Journal of Insect Physiology*. 56: 1834-1845. [Set06]

{Kovacova et al. 2013} Kovacova J; Hrbek V; Kloutvorova J; Kocourek V; Drabova L; Hajslova J. 2013. Assessment of Pesticide Residues in Strawberries Grown Under Various Treatment Regimes. *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment*. 30(12):2123-35. [Set01]

- {Kovendan et al. 2012} Kovendan K; Murugan K; Naresh Kumar A; Vincent S; Hwang JS. 2012. Bioefficacy of Larvicidal and Pupicidal Properties of *Carica papaya* (Caricaceae) Leaf Extract and Bacterial Insecticide, Spinosad, Against Chikungunya Vector, *Aedes aegypti* (Diptera: Culicidae). *Parasitology Research*. 110(2):669-78. [Set01]
- {Kumar et al. 2011} Kumar AN; Murugan K; Madhiyazhagan P; Prabhu K. 2011. Spinosad and Neem Seed Kernel Extract as Bio-Controlling Agents for Malarial Vector, *Anopheles stephensi* and Non-Biting Midge, *Chironomus circumdatus*. *Asian Pacific Journal of Tropical Medicine*. 4(8):614-8. [Set01]
- {Lawler and Dritz 2013} Lawler SP; Dritz DA. 2013. Efficacy of Spinosad in Control of Larval *Culex tarsalis* and *Chironomid* Midges, and its Nontarget Effects. *Journal of the American Mosquito Control Association*. 29(4):352-7. [Set01]
- {Lewis et al. 1998} Lewis DFV; Ioannides C; Parke DV. 1998. Cytochromes P450 and species differences in xenobiotic metabolism and activation of carcinogen. *Env Health Perspect*. 106(10): 633-641. Available at: <http://www.ehponline.org/members/1998/106p633-641lewis/lewis-full.html>. [Std]
- {Lewis et al. 2007} Lewis PA; Smitley D; Reardon R; Mastro VC. 2007. Aerial application of spinosad for EAB control in woodlots. In: Gottschalk, Kurt W., ed. Proceedings, 18th U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2007; 2007 January 9-12; Annapolis, MD. Gen. Tech. Rep. NRS-P-28. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station: 49-50. [Set00]
- {Liberacki et al. 1993} Liberacki AB; Breslin WJ; Yano BL. 1993. Data Evaluation Record (DER) for unpublished study: XDE-105: Oral gavage teratology study in Sprague-Dawley rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 435575055. 8 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]
- {Liebisch et al. 2011} Liebisch G; Hack R; Smid G. 2011. Efficacy of spinosad against the poultry red mite, *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae), in laboratory and field trials. *Zoosymposia* 6: 282-287. [Set00]
- {Lin et al. 2013} Lin Y; Jin T; Zeng L; Lu Y. 2013. Insecticide Toxicity to Oriental Fruit Fly *Bactrocera dorsalis* (Diptera: Tephritidae) is Influenced by Environmental Factors. *Insecticide Resistance and Resistance Management*. 106(1): 353-359. [Set00]
- {Liu and Li 2004} Liu S; Li QX. 2004. Photolysis of Spinosyns in Seawater, Stream Water and Various Aqueous Solutions. *Chemosphere*. 56(11):1121-7. [Set01]
- {Liu and Zhang 2012} Liu TX; Zhang Y. 2012. Side Effects of Two Reduced-Risk Insecticides, Indoxacarb and Spinosad, on Two Species of *Trichogramma* (Hymenoptera: Trichogrammatidae) on Cabbage. *Ecotoxicology*. 21(8):2254-63. [Set01]
- {Liu et al. 2004a} Liu H; Cupp EW; Micher KM; Liu N. 2004a. Insecticide Resistance and Cross-Resistance in Alabama and Florida Strains of *Culex quinquefasciatus*. *Journal of Medical Entomology*. 41(3): 408-413. [Set01 ECOTOX-88223/Toxline]
- {Liu et al. 2004b} Liu H; Cupp EW; Guo A; Liu N. 2004b. Insecticide Resistance in Alabama and Florida Mosquito Strains of *Aedes albopictus*. *Journal of Medical Entomology*. 41(5):946-52. [Set01 ECOTOX-88223/Toxline]
- {Liu et al. 2013a} Liu TX; Irungu RW; Dean DA; Harris MK. 2013a. Impacts of Spinosad and lambda;-Cyhalothrin on Spider Communities in Cabbage Fields in South Texas. *EcoToxicology*. 22(3):528-37. [Set01]
- {Liu et al. 2013b} Liu Y; Sun H; Wang S. 2013b. Dissipation and Residue of Spinosad in Zucchini under Field Conditions. *Bulletin of Environmental Contamination and Toxicology*. 91(2):256-9. [Set01]

- {MacKay et al. 2012} MacKay CS; Mattoon JS; Roberts GD; Tucker RL; Morimoto TR; Mealey KL. 2012. Evaluation of the biliary and brain distribution of technetium Tc 99m sestamibi in healthy dogs with the ABCB1 wildtype genotype before and after treatment with spinosad. *American Journal of Veterinary Research*. 73(6):814-20. [Set01]
- {Magnussen et al. 1996} Magnussen JD; Castetter SA; Rainey DP. 1996. Characterization of Spinosad Related Residues in Poultry Tissues and Eggs Following Oral Administration. 211th American Chemical Society National Meeting, New Orleans, Louisiana, USA, March 24-28, 1996. Abstracts of Papers American Chemical Society. 211(1-2): Agro 43. [Set01]
- {Mandal et al. 2009} Mandal K; Jyot G; Singh B. 2009. Dissipation Kinetics of Spinosad on Cauliflower (*Brassica Oleracea* Var. *Botrytis*. L.) Under Subtropical Conditions of Punjab, India. *Bulletin of Environmental Contamination and Toxicology*. 83(6):808-11. [Set01]
- {Mandal et al. 2013} Mandal K; Singh S; Battu RS; Singh B. 2013. An Overview of Persistence of Spinosad in Biotic and Abiotic Components of the Environment and Advances in its Estimation Techniques. *Bulletin of Environmental Contamination and Toxicology*. 90(4):405-13. [Set01]
- {Mangan and Moreno 2009} Mangan RL; Moreno AT. 2009. Honey Bee Foraging Preferences, Effects of Sugars, and Fruit Fly Toxic Bait Components. *Journal of Economic Entomology*. 102(4):1472-81. [Set01]
- {Mangini 2016} Mangini A. 2016. Unpublished Conserve® SC (spinosad) Pilot Study (2010-11) – Alex Mangini, USFS, FHP. Received via email to P. Durkin (SERA Inc.) from Alex Mangini on February 23, 2016. [FS]
- {Mansour et al. 2012} Mansour SA; Foda MS; Aly AR. 2012. Mosquitocidal Activity of Two *Bacillus* Bacterial Endotoxins Combined with Plant Oils and Conventional Insecticides. *Industrial Crops and Products*. 35(1): 44-52. [Set01 ECOTOX- 162546]
- {Marina et al. 2012} Marina CF; Bond JG; Muñoz J; Valle J; Chirino N; Williams T. 2012. Spinosad: A Biorational Mosquito Larvicide for Use in Car Tires in Southern Mexico. *Parasites and Vectors*. 5:95. [Set01]
- {Marina et al. 2014} Marina CF; Bond JG; Muñoz J; Valle J; Novelo-Gutierrez R; Williams T. 2014. Efficacy and Non-Target Impact of Spinosad, *Bti* and Temephos Larvicides for Control of *Anopheles* Spp. in an Endemic Malaria Region of Southern Mexico. *Parasites and Vectors*. 7:55. [Set00]
- {Markussen and Kristensen 2012} Markussen MD; Kristensen M. 2012. Spinosad Resistance in Female *Musca domestica* L. from a Field-Derived Population. *Pest Management Science*. 68(1):75-82. [Set01]
- {Martinou et al. 2014} Martinou AF; Seraphides N; Stavrinides MC. 2014. Lethal and Behavioral Effects of Pesticides on the Insect Predator *Macrolophus pygmaeus*. *Chemosphere*. 96:167-73. [Set00]
- {Marty et al. 1998} Marty MS; Liberacki AB; Vedula U; Breslin WJ; Yano BL. 1998. The Maternal and Developmental Toxicity of Spinosad in Sprague-Dawley Rats and New Zealand White Rabbits. *Teratology*. 57(4/5):244. [Set01]
- {Mayer et al. 2001} Mayer DF; Kovacs G; Brett BL; Brisabri BL. 2001. The effects of spinosad insecticide to adults of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi* (Hymenoptera:Apidae). *International Journal of Horticultural Science*. 7:93–97. [Set04]
- {Mayes et al. 2003} Mayes MA; Thompson GD; Husband B; Miles MM. 2003. Spinosad Toxicity to Pollinators and Associated Risk. *Reviews of Environmental Contamination and Toxicology*. 179:37-71. [Set01]
- {McCormack 2011} McCormack PL. 2011. Spinosad: In Pediculosis Capitis. *American Journal of Clinical Dermatology*. 12(5):349-53. [Set01]

{McFadden and Saunders 2004} McFadden A; Saunders C. 2004. Experimental Research Application for the use of Entrust 80W (Spinosad) on Sawflies on 45 hectares of forest in Western Newfoundland. Environmental assessment Registration pursuant to Part 10, Environmental Assessment, Section 49 of the Environmental Protection Act, 2002. Submitted to Minister of Environment and Conservation, St John's NL. A1B 4J6. Proponent: Dow AgroSciences Canada Inc., 201, 1144-29th Ave, NE, Calgary, AB T2E 7P1. 29 pp. [DER01]

{Michaud 2003} Michaud JP. 2003. Toxicity of Fruit Fly Baits to Beneficial Insects in Citrus. *Journal of Insect Science*. 3:8. [Set00]

{Milazzo et al. 1994} Milazzo DP; Kirk HD; Servinski MF; Hugo JM. 1994. Data Evaluation Record (DER) for unpublished study: XDE-105 (Lot# ACD 13651): Evaluation of the acute toxicity to the daphnid, *Daphnia magna* straus. EPA Guideline No. 72-2; Lab study No. DECO-ES-2736B. Environmental Toxicology and Chemistry Research Laboratory, Dow Chemical Co, Midland, MI. MRID No. 43414537. 9 pp. [DER01]

{Miles 2003} Miles M. 2003. The effects of spinosad, a naturally derived insect control agent to the honeybee. *Bulletin of Insectology*. 56(1): 119-124. [Set00]

{Miles and Eelen 2006} Miles M; Eelen H. 2006. The Effects of Spinosad to Beneficial Insects and Mites and its Use in IPM. *Communications in Agricultural and Applied Biological Sciences*. 71(2 Pt B):275-84. [Set01]

{Miles et al. 2002} Miles M; Mayes M; Dutton R. 2002. The Effects of Spinosad, a Naturally Derived Insect Control Agent, to the Honeybee (*Apis mellifera*). *Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet*. 67(3):611-6. [Set01]

{Miles et al. 2011} Miles MJ; Alix A; Bourgouin C; Schmitzer S. 2011. Effects of spinosad on honey bees (*Apis mellifera*): Findings from over ten years of testing and commercial use. *Julius-Kühn-Archiv*. 437: 107-114. [Set00]

{Mineau et al. 2008} Mineau P; Harding KM; Whiteside M; Fletcher MR; Garthwaite D; Knopper LD. 2008. Using reports of honeybee mortality in the field to calibrate laboratory derived pesticide risk indices. *Environmental Entomology*. 37(2): 546-554. [Set00]

{Morandin et al. 2005} Morandin LA; Winston ML; Franklin MT; Abbott VA. 2005. Lethal and Sub-Lethal Effects of Spinosad on Bumble Bees (*Bombus Impatiens* Cresson). *Pest Management Science*. 61(7):619-26. [Set00]

{Muddasir et al. 2015} Muddasir M; Ahmad Z; Fardusi MJ; Rehman A. 2015. Effects of Biological Insecticides on Predatory Spider's Population in Rice Field. *International Journal of Innovation and Applied Studies*. 11(1): 114-117. [Set00]

{Murray 1992} Murray AG. 1992. Data Evaluation Record (DER) for unpublished study: The toxicity of XDE-105 to mallards in a 14-day acute oral study. Laboratory Project ID A00991; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189. MRID 43414528. 6 pp. [DER01]

{Murray and Woolwine 1992} Murray AG; Woolwine SE. 1992. Data Evaluation Record (DER) for unpublished study: The toxicity of XDE-105 to juvenile mallards in a 5-day dietary study. Laboratory Project ID A00891; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189. MRID 43414530. 7 pp. [DER01]

{Murray et al. 1992a} Murray AG; Seacat JL; Grothe DW. 1992a. Data Evaluation Record (DER) for unpublished study: The toxicity of XDE-105 to juvenile bobwhite in a 5-day dietary study. Laboratory Project ID A00791; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189. MRID 43414531. 6 pp. [DER01]

{Murray et al. 1992b} Murray AG; Seacat JL; Grothe DW. 1992b. Data Evaluation Record (DER) for unpublished study: The toxicity of XDE-105 to bobwhite in a 14-day acute oral study. Laboratory Project ID A01091; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189. MRID 43414529. 7 pp. [DER01]

- {Musser and Shelton 2005} Musser FR; Shelton AM. 2005. The Influence of Post-Exposure Temperature on the Toxicity of Insecticides to *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Pest Management Science*. 61(5):508-10. [Set01/ECOTOX-103842]
- {Nagy 2005} Nagy KA. 2005. Field metabolic rate and body size. *Journal of Experimental Biology*. 208: 1621-1625. [Std]
- {NAS 2013} NAS (National Academy of Sciences). 2013. Assessing Risks to Endangered and Threatened Species from Pesticides. Committee on Ecological Risk Assessment under FIFRA and ESA, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council. International Standard Book Number-13: 978-0-309-28583-4. 194 pp. [Std]
- {Naveed et al. 2008} Naveed M; Salam A; Saleem AM; Sayyed AH. 2008. Effect of Foliar Applications of Some Insecticides on *Bemisia tabaci*, Predators and Parasitoids: Implications in Its Management in Pakistan. *Phytoparasitica*. 36(4): 377-387. [Set02]
- {Nebraska Forest Service 2009} Nebraska Forest Service. 2009. Insect Pests of Evergreen Trees. FH04-2009. Available at: <http://nfs.unl.edu/documents/foresthealth/insectevergreen.pdf>. [Set00]
- {Newsted and Brock 1992} Newsted JL; Brock DE. 1992. Data Evaluation Record (DER) for unpublished study: The toxicity of XDE-105 to bluegill (*Lepomis macrochirus*) in a static test system. Laboratory Project ID F00491; Lilly Research Laboratories, Greenfield, IN 46140; Submitted by DowElanco, Indianapolis, IN 46258-1189. MRID 43414534. 10 pp. [DER01]
- {Nowak et al. 2000} Nowak JT; Fettig CJ; McCravy KW; Berisford CW. 2000. Efficacy Tests and Determination of Optimal Spray Timing Values to Control Nantucket Pine Tip Moth (Lepidoptera: Tortricidae) Infestations. *Journal of Economic Entomology*. 93(6):1708-13. [Set00]
- {Nowak et al. 2001} Nowak JT; Mccravy KW; Fettig CJ; Berisford CW. 2001. Susceptibility of Adult Hymenopteran Parasitoids of the Nantucket Pine Tip Moth (Lepidoptera: Tortricidae) to Broad-Spectrum and Biorational Insecticides in a Laboratory Study. *Journal of Economic Entomology*. 94(5):1122-7. [Set01]
- {Nowak et al. 2010} Nowack JT; Asaro C; Fettig CJ; McCravy KW. 2010. Nantucket Pine Tip Moth. USDA/Forest Service, Forest Insect and Disease Leaflet 70. Document dated November 2010. Available at: <http://dnrc.mt.gov/divisions/forestry/docs/assistance/pests/fidls/070.pdf>. [Set00]
- {Paarlberg et al. 2013} Paarlberg TE; Wiseman S; Trout CM; Kee EA; Snyder DE. 2013. Safety and Efficacy of Spinosad Chewable Tablets for Treatment of Flea Infestations of Cats. *Journal of the American Veterinary Medical Association*. 242(8):1092-8. [Set01]
- {Pauli et al. 2000} Pauli BD; Perrault JA; Money SL. 2000. RATL: A Database of Reptile and Amphibian Toxicology Literature. National Wildlife Research Centre 2000, Canadian Wildlife Service, Environmental Conservation Branch, Technical Report Series Number 357. Available at: <http://dsp-psd.communication.gc.ca/Collection/CW69-5-357E.pdf>. [Std]
- {Penagos et al. 2005} Penagos DI; Cisneros J; Hernandez O; Williams, T. 2005. Lethal and sublethal effects of the naturally derived insecticide spinosad on parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biocontrol Science and Technology*. 15:81-95. [Set03]
- {Perez et al. 2007} Perez CM; Marina CF; Bond JG; Rojas JC; Valle J; Williams T. 2007. Spinosad, a Naturally Derived Insecticide, for Control of *Aedes aegypti* (Diptera: Culicidae): Efficacy, Persistence, and Elicited Oviposition Response. *Journal of Medical Entomology*. 44(4):631-8. [Set01]
- {Peusens and Belian 2012} Peusens G; Belian T. 2012. Life Cycle and Control of the Forest Bug *Pentatoma rufipes* L. in Organically Managed Pear Orchards. *Communications in Agricultural and Applied Biological Sciences*. 77(4):663-6. [Set01]

- {Pietrantonio and Benedict 1999} Pietrantonio PV; Benedict JH. 1999. Effect of New Cotton Insecticide Chemistries, Tebufenozide, Spinosad and Chlorfenapyr, on *Orius insidiosus* and Two Cotesia Species. Southwestern Entomologist. 24 (1): 21-29. [Set01]
- {Pineda et al. 2007} Pineda S; Schneider M; Smagghe G; Martinez A; Del Estal P; Uela EV; Valle J; Budia F. 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). Journal of Economic Entomology. 100(3):773-780. [Set03]
- {Piner and Šner 2013} Piner P; Šner N. 2013. Oxidative Stress and Apoptosis Was Induced by Bio-Insecticide Spinosad in the Liver of *Oreochromis niloticus*. Environmental Toxicology and Pharmacology. 36(3):956-63. [Set01]
- {Piner and Uner 2012} Piner P; Uner N. 2012. *In Vivo* Acetylcholinesterase Inhibition in the Tissues of Spinosad Exposed *Oreochromis niloticus*. Environmental Toxicology and Pharmacology. 34(2):473-7. [Set01]
- {Piner and Uner 2014} Piner P; Uner N. 2014. Organic Insecticide Spinosad Causes *in vivo* Oxidative Effects in the Brain of *Oreochromis niloticus*. Environmental Toxicology. 29(3):253-60. [Set01]
- {Posthuma et al. 2002} Posthuma L; Suter GW; Trass TP. 2002. Species Sensitivity Distributions. Lewis Publishers, Boca Raton, Florida, 587 pp. Available at: <http://www.crcnetbase.com/isbn/9781420032314> . [Std]
- {Potter et al. 1990} Potter DA; Buxton MC; Redmond CT; Patterson CG; Powell AJ. 1990. Toxicity of Pesticides to Earthworms (Oligochaeta: Lumbricidae) and Effect on Thatch Degradation in Kentucky Bluegrass Turf. Journal of Economic Entomology. 83(6): 2362-2369. [Std-Compendia]
- {Potter et al. 1994} Potter DA; Spicer PG; Redmond CT. Powell AJ. 1994. Toxicity of Pesticides to Earthworms in Kentucky Bluegrass Turf. Bulletin of Environmental Contamination and Toxicology. 52:176-181. [Std-Compendia]
- {Pridgeon et al. 2008} Pridgeon JW; Pereira RM; Becnel JJ; Allan SA; Clark GG; Linthicum KJ. 2008. Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 Pesticides with Different Modes of Action. Journal of Medical Entomology. 45(1):82-7. [Set01]
- {Quaranta et al. 2009} Quaranta A; Bellantuono V; Cassano G; Lippe C. 2009. Why Amphibians Are More Sensitive than Mammals to Xenobiotics. PLoS ONE. 4: (11): 1-4. [Std]
- {Rabea et al. 2010} Rabea EI; Nasr HM; Badawy ME. 2010. Toxic Effect and Biochemical Study of Chlorfluazuron, Oxymatrine, and Spinosad on Honey Bees (*Apis Mellifera*). Archives of Environmental Contamination and Toxicology. 58(3):722-32. [Set01]
- {Rahman et al. 2011} Rahman T; Spafford H; Broughton S. 2011. Compatibility of Spinosad with Predaceous Mites (Acari) Used to Control *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Pest Management Science. 67(8):993-1003. [Set01]
- {Reichle et al. 1973} Reichle DE, Goldstein RA; Van Hook RI; Dodson DJ. 1973. Analysis of Insect Consumption in a Forest Canopy. Ecology. 54: 1076-1084. [Std]
- {Redoan et al. 2013} Redoan ACM; Carvalho GAC; Cruz I; et al. 2013. Physiological selectivity of insecticides to adult of *Doru luteipes* (Scudder, 1876) (Dermaptera: Forficulidae). Revista Ciência Agronômica, 44(4) 842-850. [Set05]
- {Reid 2006} Reid FA. 2006. A Field Guide to Mammals of North America North of Mexico. Fourth Edition. The Peterson Field Guide Series. Houghton Mifflin Company, Boston MA. 570 pp. [Std]
- {Rimoldi et al. 2012} Rimoldi F; Schneider MI; Ronco AE. 2012. Short and Long-Term Effects of Endosulfan, Cypermethrin, Spinosad, and Methoxyfenozide on Adults of *Chrysoperla externa* (Neuroptera: Chrysopidae). Journal of Economic Entomology. 105(6):1982-7. [Set01]

- {Rinkevich and Scott 2013} Rinkevich FD; Scott JG. 2013. Limitations of RNAi of $\alpha 6$ Nicotinic Acetylcholine Receptor Subunits for Assessing the in Vivo Sensitivity to Spinosad. *Insect Science*. 20(1):101-8. Available at: <http://scott.entomology.cornell.edu/170.pdf> . [Set00]
- {Romi et al. 2006} Romi R; Proietti S; Di Luca M; Cristofaro M. 2006. Laboratory Evaluation of the Bioinsecticide Spinosad for Mosquito Control. *Journal of the American Mosquito Control Association*. 22(1):93-6. [Set01]
- {Rortais et al. 2005} Rortais A; Arnold G; Halm MP; Touffet-Briens F. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie (Celle)* 36: 71-83. [Set00]
- {Rothwell et al. 2005} Rothwell JT; Carson J; Sherwood N; Shields R; Hacket K; Burnett T. 2005. Residues of Spinosad in the Tissues of Sheep After Aerosol Treatment of Blowfly Myiasis. *Australian Veterinary Journal*. 83(3):154-6. [Set01]
- {Ryberg et al. 2011} Ryberg KR; Vecchia AV; Martin JD; Gilliom RJ. 2011. Trends in Pesticide Concentrations in Urban Streams in the United States, 1992–2008. USGS National Water-Quality Assessment Program. Available at: <http://pubs.usgs.gov/sir/2010/5139/>. [Std]
- {Sage 1982} Sage RD. 1982. Wet and Dry-weight Estimates of Insects and Spiders Based on Length. *The American Midland Naturalist*. 108(2): 407-411. [Std]
- {Sagri et al. 2014} Sagri E; Reczko M; Gregoriou ME; Tsoumani KT; Zygouridis NE; Salpea KD; Zalom FG; Ragoussis J; Mathiopoulos KD. 2014. Olive Fly Transcriptomics Analysis Implicates Energy Metabolism Genes in Spinosad Resistance. *BMC Genomics*. 15:714. [Set01]
- {Saikumar and Venkatachalam 2009} Saikumar P; Venkatachalam MA. 2009. Apoptosis and Cell Death. Chapter 2 in *Basic Concepts of Molecular Pathology*, Volume 2 of the series *Molecular Pathology Library*. pp 29-40. Available at: <http://www.springer.com/us/book/9780387896250>. [Set04]
- {Salgado 1998} Salgado VL. 1998. Studies on the Mode of Action of Spinosad: Insect Symptoms and Physiological Correlates. *Pesticide Biochemistry and Physiology*. 60 (2): 91-102. [Set01]
- {Salgado et al. 1998} Salgado VL; Sheets JJ; Watson GB; Schmidt AL. 1998. Studies on the Mode of Action of Spinosad: The Internal Effective Concentration and the Concentration Dependence of Neural Excitation. *Pesticide Biochemistry and Physiology*. 60 (2): 103-110. [Set01]
- {Sample and Arenal 1999} Sample BE; Arenal CA. 1999. Allometric Models for Interspecies Extrapolation of Wildlife Toxicity Data. *Bulletin of Environmental Contamination and Toxicology*. 62 (6): 653-663. [Std]
- {Sanchez et al. 2012} Sanchez D; De J Solórzano E; Liedo P; Vandame R. 2012. Effect of the Natural Pesticide Spinosad (Gf-120 Formulation) on the Foraging Behavior of *Plebeia moureana* (Hymenoptera: Apidae). *Journal of Economic Entomology*. 105(4):1234-7. [Set01]
- {Santis et al. 2012} Santis EL; Hernández LA; Martínez AM; Campos J; Figueroa JI; Lobit P; Chavarrieta JM; Viñuela E; Smagghe G; Pineda S. 2012. Long-Term Foliar Persistence and Efficacy of Spinosad Against Beet Armyworm Under Greenhouse Conditions. *Pest Management Science*. 68(6):914-21. [Set01]
- {Sayyed et al. 2008} Sayyed AH; Saeed S; Noor-Ul-Ane M; Crickmore N. 2008. Genetic, Biochemical, and Physiological Characterization of Spinosad Resistance in *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology*. 101(5):1658-66. [Set01]
- {Schneider et al. 2003} Schneider MI; Smagghe G; Gobbi A; Viñuela E. 2003. Toxicity and Pharmacokinetics of Insect Growth Regulators and Other Novel Insecticides on Pupae of *Hyposoter didmator* (Hymenoptera: Ichneumonidae), a Parasitoid of Early Larval Instars of Lepidopteran Pests. *Journal of Economic Entomology*. 96(4):1054-65. [Set01]

{Schneider et al. 2004} Schneider MI; Smaghe G; Pineda S; Vinuela E. 2004. Action of Insect Growth Regulator Insecticides and Spinosad on Life History Parameters and Absorption in Third-Instar Larvae of the Endoparasitoid *Hyposoter didymator*. *Biological Control*. 31(2): 189-198. [Set00]

{Schoonover and Larson 1995} Schoonover JR; Larson LL. 1995. Laboratory Activity of Spinosad on Non-Target Beneficial Arthropods 1994. *Arthropod Management Tests*. 20: 357. At: <http://www.entsoc.org/Pubs/Periodicals/AMT>. [Set00]

{Schrickx 2014} Schrickx JA. 2014. Spinosad Is a Potent Inhibitor of Canine P-Glycoprotein. *The Veterinary Journal*. 200(1):195-6. [Set01]

{Scott 1998} Scott JG. 1998. Toxicity of Spinosad to Susceptible and Resistant Strains of House Flies, *Musca domestica*. *Pesticide Science*. 54 (2): 131-133. [Set01]

{Scott-Dupree et al. 2009} Scott-Dupree CD; Conroy L; Harris CR. 2009. Impact of Currently Used or Potentially Useful Insecticides for Canola Agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *Journal of Economic Entomology*. 102(1):177-82. [Set01]

{Semiz et al. 2006} Semiz G; Cetin H; Isik K; Yanikoglu A. 2006. Effectiveness of a Naturally Derived Insecticide, Spinosad, Against the Pine Processionary Moth *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Thaumetopoeidae) Under Laboratory Conditions. *Pest Management Science*. 62(5):452-5. [Set01]

{SERA 2007a} SERA (Syracuse Environmental Research Associates, Inc.). 2007a. Gleams-Driver User Guide (Version 1.8). SERA TR 07-52-05-08a. Report dated December 31, 2007. Available at: www.sera-inc.com. [Std]

{SERA 2007b} SERA (Syracuse Environmental Research Associates, Inc.). 2007b. Aminopyralid Human Health and Ecological Risk Assessment – FINAL REPORT. SERA TR-052-04-04a. Report dated June 28, 2007. Available at: <http://www.fs.fed.us/foresthealth/pesticide/risk.shtml>. [Std]

{SERA 2009a} SERA (Syracuse Environmental Research Associates, Inc.). 2009a. Dinotefuran, Human Health and Ecological Risk Assessment. SERA TR-052-18-03b. Document dated April 24, 2009. Available at: <http://www.fs.fed.us/foresthealth/pesticide/risk.shtml>. [Std]

{SERA 2010a} SERA (Syracuse Environmental Research Associates, Inc.). 2010a. Glyphosate, Human Health and Ecological Risk Assessment, Final Report. SERA TR-052-22-03a. Document dated November 29, 2010. Available at: <http://www.fs.fed.us/foresthealth/pesticide/risk.shtml>. [Std]

{SERA 2011a} SERA (Syracuse Environmental Research Associates, Inc.). 2011a. WorksheetMaker Version 6.00, User Guide. SERA TR-052-20-01b. Document dated December 21, 2011. Syracuse Environmental Research Associates, Inc., Fayetteville, NY. Available at www.sera-inc.com. [Std]

{SERA 2011b} SERA (Syracuse Environmental Research Associates, Inc.). 2011b. Memorandum on Release of Gleams-Driver 1.9.3. Memo dated November 6, 2011. Available at: www.sera-inc.com. [Std]

{SERA 2014a} SERA (Syracuse Environmental Research Associates, Inc.). 2014a. Preparation of Environmental Documentation and Risk Assessments, SERA MD-2014-02b. Document dated November 17, 2014. Syracuse Environmental Research Associates, Inc., Manlius, NY. Available at www.sera-inc.com. [Std]

{SERA 2014b} SERA (Syracuse Environmental Research Associates, Inc.). 2014b. Reassessment of Worker Exposure Rates – FINAL REPORT. SERA TR-056-06-02b. Document dated November 17, 2014. Syracuse Environmental Research Associates, Inc., Manlius, NY. Available at: <http://www.fs.fed.us/foresthealth/pesticide/risk.shtml>. [Std]

{SERA 2015} SERA (Syracuse Environmental Research Associates, Inc.). 2015. Imidacloprid: Human Health and Ecological Risk Assessment, FINAL REPORT. SERA TR-056-09-02c. Document dated August 31, 2015. Syracuse Environmental Research Associates, Inc., Manlius, NY. Available at: <http://www.fs.fed.us/foresthealth/pesticide/risk.shtml>. [Std]

- {Sharma et al. 2007} Sharma A; Srivastava A; Ram B; Srivastava PC. 2007. Dissipation Behaviour of Spinosad Insecticide in Soil, Cabbage and Cauliflower under Subtropical Conditions. *Pest Management Science*. 63(11):1141-5. [Set01]
- {Sharma et al. 2008} Sharma A; Srivastava A; Ram B; Srivastava PC. 2008. Dissipation Behaviour of Spinosad Insecticide in Chilli and Soil. *Asian Journal of Water, Environment and Pollution*. 5:49–52. [Set02]
- {Sherman et al. 2010} Sherman JG; Paul AJ; Firkins LD. 2010. Evaluation of the Safety of Spinosad and Milbemycin 5-Oxime Orally Administered to Collies with the Mdr1 Gene Mutation. *American Journal of Veterinary Research*. 71(1):115-9. [Set01]
- {Shi et al. 2011} Shi J; Zhang L; Gao X. 2011. Characterisation of Spinosad Resistance in the Housefly *Musca domestica* (Diptera: Muscidae). *Pest Management Science*. 67(3):335-40. [Set01]
- {Shmidt and Levitt 2012} Shmidt E; Levitt J. 2012. Dermatologic Infestations. *International Journal of Dermatology*. 51(2):131-41. [Set01]
- {Sibley 2000} Sibley DA. 2000. *The Sibley Guide to Birds*. National Audubon Society, Chanticleer Press Edition. Alfred A. Knopf Inc., New York. 545 pp. [Std]
- {Singh and Battu 2012} Singh S; Battu RS. 2012. Dissipation kinetics of spinosad in cabbage (*Brassica oleracea* L.var. capitata). *Toxicology and Environmental Chemistry*. 94:319–326. [Set02]
- {Slaninov et al. 2009} Slaninova A; Smutna M; Modra H; Svobodova Z. 2009. A review: Oxidative stress in fish induced by pesticides. *Neuroendocrinology Letters*. 30(Suppl. 1): 2-12. [Std]
- {Snyder et al. 2013} Snyder DE; Meyer KA; Wiseman S; Trout CM; Young DR. 2013. Speed of Kill Efficacy and Efficacy of Flavored Spinosad Tablets Administered Orally to Cats in a Simulated Home Environment for the Treatment and Prevention of Cat Flea (*Ctenocephalides felis*) Infestations. *Veterinary Parasitology*. 196(3-4):492-6. [Set01]
- {Sparks and Nauen 2015} Sparks TC; Nauen R. 2015. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*. 121: 122-128. [Std]
- {Sparks et al. 1998} Sparks TC; Thompson GD; Kirst HA; Hertlein MB; Mynderse JS; Turner JR; Worden TV. 1998. Fermentation-derived insect control agents: The spinosyns. *Biopesticides: Use and Delivery*. 5: 171-188. [Set01 – EFED 2011a]
- {Spencer and Yano 1995} Spencer PJ; Yano BL. 1995. Data Evaluation Record (DER) for unpublished study: XDE-105: Chronic neurotoxicity study in Fischer rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43710503. 9 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]
- {Stark 2005} Stark, J.D., 2005. How closely do acute lethal concentration estimates predict effects of toxicants on populations? *Integrated Environmental Assessment and Management*. 1(2): 109–113. [Set05]
- {Stark and Vargas 2003} Stark JD; Vargas RI. 2003. Demographic Changes in *Daphnia pulex* (Leydig) After Exposure to the Insecticides Spinosad and Diazinon. *Ecotoxicology and Environmental Safety*. 56(3):334-8. [Set01]
- {Stark et al. 2004} Stark JD; Vargas R; Miller N. 2004. Toxicity of Spinosad in Protein Bait to Three Economically Important Tephritid Fruit Fly Species (Diptera: Tephritidae) and Their Parasitoids (Hymenoptera: Braconidae). *Journal of Economic Entomology*. 97(3):911-5. [Set01]
- {Stebbins et al. 2002} Stebbins KE; Bond DM; Novilla MN; Reasor MJ. 2002. Spinosad insecticide: Subchronic and chronic toxicity and lack of carcinogenicity in CD-1 Mice. *Toxicological Sciences*. 65: 276-287.

- {Stebbins et al. 2002} Stebbins KE; Bond DM; Novilla MN; Reasor MJ. 2002. Spinosad Insecticide: Subchronic and Chronic Toxicity and Lack of Carcinogenicity in CD-1 Mice. *Toxicological Sciences*. 65(2):276-87. [Set01]
- {Stoliar and Lushchak 2012} Stoliar OB; Lushchak VL. 2012. Environmental Pollution and Oxidative Stress in Fish, Oxidative Stress - Environmental Induction and Dietary Antioxidants, Dr. Volodymyr Lushchak (Ed.), ISBN: 978-953-51-0553-4, InTech, Available from: <http://www.intechopen.com/books/oxidative-stress-environmentalinduction-and-dietary-antioxidants/environmental-pollution-and-oxidative-stress-in-fish>. [Std]
- {Stough 2012} Stough D. 2012. Efficacy and Safety of Spinosad Cream Rinse for Head Lice-Reply. *Archives of Dermatology*. 148(9):1070. [Set01]
- {Stough et al. 2009} Stough D; Shellabarger S; Quiring J; Gabrielsen Aa Jr. 2009. Efficacy and Safety of Spinosad and Permethrin Creme Rinses for Pediculosis Capitis (Head Lice). *Pediatrics*. 124(3):e389-95. [Set01]
- {Strek and Spaan 1997} Strek G; Spaan WP. 1997. Wind erosion control with crop residues in the Sahel. *Soil Sci. Soc. Am. J.* 61(3): 911-917. [Std]
- {Strek and Stein 1997} Strek G; Stein A. 1997. Mapping wind-blown mass transport by modeling variability in space and time. *Soil Sci. Soc. Am. J.* 61(1): 232-239. [Std]
- {Studebaker and Kring 2003} Studebaker G.E., and T.J. Kring. 2003. Effects of Insecticides on *Orius insidiosus* (Hemiptera: Anthracoridae), Measured by Field, Greenhouse and Petri Dish Bioassays. *Florida Entomologist*. 86(2): 178-185. [Set00]
- {Su and Cheng 2014a} Su T; Cheng ML. 2014a. Cross Resistances in Spinosad-Resistant *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology*. 51(2):428-35. [Set01]
- {Su and Cheng 2014b} Su T; Cheng ML. 2014b. Laboratory Selection of Resistance to Spinosad in *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology*. 51(2):421-7. [Set01]
- {Su et al. 2011} Su TY; Lin JL; Lin-Tan DT; Tseng HH; Yen TH. 2011. Human Poisoning with Spinosad and Flocicamid Insecticides. *Human and Experimental Toxicology*. 30(11):1878-81. [Set01]
- {Su et al. 2014} Su T; Cheng ML; Thieme J. 2014. Laboratory and Field Evaluation of Spinosad Formulation Natular T30 against Immature *Culex* Mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*. 51(4):837-44. [Set01]
- {Suter and Bartell 1993} Suter G; Bartell S. 1993. Ecosystem-Level Effects. Chapter 9, pp. 275-308 in: *Ecological Risk Assessment*. Lewis Publishers, Chelsea, Michigan. ISBN 0-87371-875-5. 538 pp. [Std]
- {Tao 2014} Tao HL. 2014. Height, weight, and entry earnings of female graduates in Taiwan. *Econ Hum Biol*. 13:85-98. doi: 10.1016/j.ehb.2013.12.006. Epub 2014 Jan 2. Available at: http://www.econ.ndhu.edu.tw/ezfiles/47/1047/attach/8/pta_23426_6802008_96098.pdf. [Set00]
- {Thomas and Mangan 2005} Thomas DB; Mangan RL. 2005. Nontarget Impact of Spinosad Gf-120 Bait Sprays for Control of the Mexican Fruit Fly (Diptera: Tephritidae) in Texas Citrus. *Journal of Economic Entomology*. 98(6):1950-6. [Set01]
- {Thompson 2015} Thompson J. 2015. Extrapolation of Acute Toxicity Across Bee Species. *Integrated Environmental Assessment and Management*. doi: 10.1002/ieam.1737. [Epub ahead of print]. [Std]
- {Thompson et al. 2000} Thompson GD; Dutton R; Sparks TC. 2000. Spinosad – a case study: an example from a natural products discovery programme. *Pest Management Science*. 56:696–702. [Set03]
- {Thompson et al. 2002a} Thompson DG; Harris BJ; Lanteigne LJ; Buscarini TM; Chartrand DT. 2002a. Fate of Spinosad in Litter and Soils of a Mixed Conifer Stand in the Acadian Forest Region of New Brunswick. *Journal of Agricultural and Food Chemistry*. 50(4):790-5. [Set01]

{Thompson et al. 2002b} Thompson DG; Harris BJ; Buscarini TM; Chartrand DT. 2002b. Fate of Spinosad in Litter and Soils of a White Spruce Plantation in Central Ontario. *Pest Management Science*. 58(4):397-404. [Set01]

{Thompson et al. 2015} Thompson GD; Hutchins SH; Sparks TC. 2015. Development of Spinosad and Attributes of a New Class of Insect Control Products, Radcliff's IPM World Textbook Website, University of Minnesota. Available at: <http://ipmworld.umn.edu/thompson-spinosad>. [Set00]

{Tom, et al. 2015} Tom, HV; Barbosa WF; Martins GF; Guedes RN. 2015. Spinosad in the Native Stingless Bee *Melipona quadrifasciata*: Regrettable Non-Target Toxicity of a Bioinsecticide. *Chemosphere*. 124:103-9. [Set00]

{Tome et al. 2014} Tome HV; Pascini TV; Dangelo RAC; Guedes RNC; Martins GF. 2014. Survival and Swimming Behavior of Insecticide-Exposed Larvae and Pupae of the Yellow Fever Mosquito *Aedes aegypti*. *Parasites and Vectors*. 7(1): 17 p. [Set01/EXOTOX- 168322]

{Tomkins et al. 1999} Tomkins AR; Holland PT; Thomson C; Willson DJ; Malcolm CP. 1999. Residual life of spinosad on kiwifruit—biological and chemical studies. *Proceedings 52nd New Zealand Plant Protection Conference*. pp 94–97. [Set02]

{Tomlin 2004} Tomlin C. 2004. Entry 737 spinosad. *The e-Pesticide Manual, Thirteenth Edition, Crop Protection Publications; British Crop Protection Council*. Available at: <http://www.bcpbookshop.co.uk>. [Std]

{U.S. EPA/OCSPP 2012a} U.S. EPA/OCSPP (U.S. Environmental Protection Agency/Office of Chemical Safety and Pollution Prevention). 2012a. Ecological Effects Test Guidelines OCSPP 850.2100: Avian Acute Oral Toxicity Test. Document dated January 2012. Available at: <https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-850-ecological-effects-test-guidelines>. [Std]

{U.S. EPA/OCSPP 2012b} U.S. EPA/OCSPP (U.S. Environmental Protection Agency/Office of Chemical Safety and Pollution Prevention). 2012b. Ecological Effects Test Guidelines, OCSPP 850.3020: Honey Bee Acute Contact Toxicity Test. EPA 712-C-019, January 2012. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0154-0007>. [Std]

{U.S. EPA/OCSPP 2012c} U.S. EPA/OCSPP (U.S. Environmental Protection Agency/Office of Chemical Safety and Pollution Prevention). 2012c. Ecological Effects Test Guidelines, OCSPP 850.3100: Earthworm Subchronic Toxicity Test. EPA 712-C-016, January 2012. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0154-0019>. [Std]

{U.S. EPA/OCSPP 2012d} U.S. EPA/OCSPP (U.S. Environmental Protection Agency/Office of Chemical Safety and Pollution Prevention). 2012d. Ecological Effects Test Guidelines, OCSPP 850.4900: Terrestrial Soil-Core Microcosm Test. EPA 712-C-003, January 2012. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0154-0007>. [Std]

{U.S. EPA/OCSPP 2015} U.S. EPA/OCSPP (U.S. Environmental Protection Agency/ Office of Chemical Safety and Pollution Prevention). 2015. OCSPP Harmonized Test Guidelines. OPPTS Harmonized Test Guidelines - Master List. Last Updated February, 2013. Available at: https://www.epa.gov/sites/production/files/2015-11/documents/ocspp-testguidelines_masterlist-2015-11-19.pdf. [Std]

{U.S. EPA/OPP 1999a} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 1999a. Pesticide Fact Sheet: Spinosad. Document dated July 19, 1999. Available at: http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-110003_19-Jul-99.pdf. [Set00]

{U.S. EPA/OPP 2008} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2008. FQPA Index Reservoir Screening Tool, Version 1.1.1. March 26, 2008 Available at: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/first-version-111-description>. [Std]

{U.S. EPA/OPP 2009a} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2009a. Pesticide Fact Sheet: Spinetoram. Document dated October 2009. Available at: http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_G-4674_01-Oct-09.pdf. [Set00]

{U.S. EPA/OPP 2011a} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2011a. BEAD Chemical Profile for Registration Review: Spinetoram (110008 and 110009). [Set00]

{U.S. EPA/OPP 2011b} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2013b. Spinosad and Spinetoram Summary Document, Registration Review: Initial Docket. Document dated September 2011. [Set00]

{U.S. EPA/OPP 2012a} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2012a. Spinosad and Spinetoram, Final Work Plan (FWP) For Registration Review. Document dated March 2012. [Set00]

{U.S. EPA/OPP 2015a} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2015a. Reduced Risk and Organophosphate Alternative Decisions for Conventional Pesticides. List updated on 3/19/2015. Available at: <http://www2.epa.gov/pesticide-registration/reduced-risk-and-organophosphate-alternative-decisions-conventional>. [Set00]

{U.S. EPA/OPP 2015b} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2015b. Label Review Manual. Updated 2015. Available at: <http://www2.epa.gov/sites/production/files/2015-08/documents/lrm-chap1-18-aug-2015.pdf>. [Std]

{U.S. EPA/OPP 2015c} U.S. EPA/OPP (U.S. Environmental Protection Agency/Office of Pesticide Programs). 2015c. Spinosad; Pesticide Tolerances. Federal Register. 80(248):80665-80672. [Set00]

{U.S. EPA/OPP/EFED 2005} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2005. EFED Risk Assessment for the Proposed IR-4 Uses of Spinosad Products on production of non-grass animal feed, legumes grown for seed, mint, green onion, pasture and rangeland. Report dated September 27, 2005. [Set00]

{U.S. EPA/OPP/EFED 2009a} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2009a. EFED Risk Assessment for the Proposed IR-4 Use of the Spinosad product Entrust® on Pomegranate and Dates, PC Code: 110003 DP Barcodes: 358851 and 358852. Report dated May 4, 2009. [Set00]

{U.S. EPA/OPP/EFED 2009b} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2009b. Drinking Water Assessment for the Use of Spinosad on Dates and Pomegranate under the IR4 Petition. Report dated June 30, 2009. [Set00]

{U.S. EPA/OPP/EFED 2010a} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2010a. Environmental Fate and Ecological Risk Assessment for the Proposed New Use of Spinosad as a Commercial Seed Treatment. Document dated 9/13/2010. Available at: <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2009-0081-0233>. [Set00]

{U.S. EPA/OPP/EFED 2011a} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2011a. Registration Review-Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking Water Assessments for Spinosad and Spinetoram (PC Codes 110003 and 110008/110009; D387961). Document signed 8/18/2011. [Set00]

{U.S. EPA/OPP/EFED 2012a} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2012a. EPA Response to Comments Related to the Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking Water Assessments for Spinosad and Spinetoram (PC Codes 110003 and 110008/110009; D396855). Document signed 2/21/2012. [Set00]

{U.S. EPA/OPP/EFED 2012b} U.S. EPA/OPP/EFED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Environmental Fate and Effects Division). 2012b. Guidance for Using PRZM-GW in Drinking Water Exposure Assessments. Document dated October 15, 2012. Document available at: http://www.epa.gov/oppefed1/models/water/przm_gw/wqtt_przm_gw_guidance.htm. [Std]

{U.S. EPA/OPP/HED 1997a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 1997a. Review of Toxicology Data Submitted by DowElanco to Support Registration of Spinosad for Use on Cotton as an Insecticide. Document dated May 30, 1997. [Set00]

{U.S. EPA/OPP/HED 1997b} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 1997b. Spinosad – 110003. Health Effects Division Risk Characterization Document for Use of Spinosad (Tracer®) on Cotton (PP#6F04735). Document dated May 30, 1997. [Set00]

{U.S. EPA/OPP/HED 2007a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2007a. Spinetoram and Spinosad - Chronic Dietary Exposure and Risk Assessments. PP#: 5F7006. PC Codes: Spinetoram (110008 and 110009) and Spinosad (110003). DP #: 337531. Decision #: 362634.. Document dated September 12, 2007. [Set00]

{U.S. EPA/OPP/HED 2009a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2009a. Spinosad and Spinetoram. Human-Health Risk Assessment for Application of Spinosad to Date and Pomegranate and Spinetoram to Pineapple, Date, Pomegranate, Hops, and Spices (Crop Subgroup 19B, except black pepper). Document dated June 9, 2009. [Set00]

{U.S. EPA/OPP/HED 2009b} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2009b. Spinosad/Spinetoram. Dietary Risk Assessment in Support of the Requested use on Date, Pomegranate, Pineapple, Spice (Subgroup 19B; except black pepper), Hop, Tree Nuts (Crop Group 14), and/or Pistachio. Document dated May 27, 2009. [Set00]

{U.S. EPA/OPP/HED 2010a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2010a. Spinosad/Spinetoram. Dietary Risk Assessment in Support of the Proposed Kitten/Cat Spot-on Use. Document dated August 18, 2010. [Set00]

{U.S. EPA/OPP/HED 2010b} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2010b. Spinosad and Spinetoram. Human-Health Risk Assessment for Direct-Spray Use on Poultry and Discontinuation by Voluntary Cancellation of the Cattle Pour-On and Direct Cattle Spray Registrations. Document dated March 25, 2010. [Set00]

{U.S. EPA/OPP/HED 2011a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2011a. Spinosad and Spinetoram: Human Health Assessment Scoping Document in Support of Registration Review. Document dated August 9, 2011. [Set00]

{U.S. EPA/OPP/HED 2011b} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2011b. Spinosad and Spinetoram: Review of Human Incidents. Document dated May 31, 2011. [Set00]

{U.S. EPA/OPP/HED 2012a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2012a. Spinosad and Spinetoram. Public Comments Regarding the Health Effects Division's (HED's) Human-Health Assessment Scoping Document in Support of Registration Review (09-AUG-2011; D387988). HED's Response to Public Comments. Document dated February 8, 2012. [Set00]

{U.S. EPA/OPP/HED 2014a} U.S. EPA/OPP/HED (U.S. Environmental Protection Agency/Office of Pesticide Programs/Health Effects Division). 2014a. Flonicamid: Human Health Assessment Scoping Document in Support of Registration Review. Document dated November 24, 2014. Available at: <https://www.regulations.gov/#!documentDetail:D=EPA-HQ-OPP-2014-0777-0008>. [Set00]

{U.S. EPA/OPPTS 1998a} U.S. EPA/OPPT (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 1998a. Health Effects Test Guidelines, OPPTS 870.7485, Metabolism and Pharmacokinetics. EPA 712-C-98-244. Document dated August 1998. Available at: <http://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines>. [Std]

{U.S. EPA/OPPTS 1998b} U.S. EPA/OPPT (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 1998b. Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity. EPA 712-C-98-193. Document dated August 1998. Available at: <https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines>. [Std]

{U.S. EPA/OPPTS 1998c} U.S. EPA/OPPT (U.S. Environmental Protection Agency/Office of Pollution Prevention and Toxic Substances). 1998c. Health Effects Test Guidelines, OPPTS 870.3465, 90-Day Inhalation Toxicity. EPA 712-C-98-204. Document dated August 1998. Available at: <https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines>. [Std]

{U.S. EPA/OPPTS 2000} U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides, and Toxic Substances). 2000. Health Effects Test Guidelines, OPPTS 870.3550, Reproduction/Developmental Toxicity Screening Test. EPA712-C-00-367. July 2000. [Std]

{U.S. EPA/OPPTS 2003} U.S. EPA/OPPTS (U.S. Environmental Protection Agency/Office of Prevention, Pesticides, and Toxic Substances). 2003a. Health Effects Test Guidelines, OPPTS 870.2600, Skin Sensitization. EPA 712-C-03-197, Report dated March 2003. Available at: https://ntp.niehs.nih.gov/icevam/suppdocs/feddocs/epa/epa_870r_2600.pdf. [Std]

{U.S. EPA/ORD 1992} U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1992. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Interim Report. Exposure Assessment Group, Office of Health and Environmental Assessment, Washington, DC. Available at: <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12188>. [Std]

{U.S. EPA/ORD 1993} U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 1993. Wildlife Exposure Factors Handbook. Volumes 1 and 2. EPA/600/R-93/187a,b. Pagination not continuous. NTIS PB94-174778 and PB94-174779. Available at: <http://rais.ornl.gov/homepage>. [Std]

{U.S. EPA/ORD 2007} U.S. EPA/ORD (U.S. Environmental Protection Agency/Office of Research and Development). 2007. Dermal Exposure Assessment: A Summary of EPA Approaches. EPA/600/R-07/040F. Report dated September 2007. Available at <http://www.epa.gov/ncea>. [Std]

{Uggini et al. 2012} Uggini GK; Patel PV; Balakrishnan S. 2012. Embryotoxic and Teratogenic Effects of Pesticides in Chick Embryos: A Comparative Study Using Two Commercial Formulations. Environmental Toxicology. 27(3):166-74. [Set01]

{USDA/APHIS 1999} USDA/APHIS (U.S. Department of Agriculture/Animal and Plant Health Inspection Service). 1999. Spinosad Bait Spray Applications. Human Health Risk Assessment. Document dated March 1999. [Set00]

{USDA/APHIS 2003} USDA/APHIS (U.S. Department of Agriculture/Animal and Plant Health Inspection Service). 2003. Spinosad Bait Spray Applications: Nontarget Rick Assessment. Document dated October 2003. Available at: https://www.aphis.usda.gov/plant_health/ea/downloads/spinosad-non-target-ra.pdf. [Set00]

{USDA/APHIS 2011} USDA/APHIS (U.S. Department of Agriculture/Animal and Plant Health Inspection Service). 2011. Ecological Risk Assessment for European Grapevine Moth Control in Nurseries and Noncommercial Applications. Document dated March 2011. Available at: https://www.aphis.usda.gov/plant_health/ea/downloads/2011/EGVMPesticideEnvironmentalRiskAssessment.pdf. [Set00]

- {USDA/APHIS 2014} USDA/APHIS (U.S. Department of Agriculture/Animal and Plant Health Inspection Service). 2014. Human Health and Ecological Risk Assessment for STATIC™ Spinosad ME Bait Applications. Document dated March 2014. [Set00]
- {USDA/NRCS 1999} USDA/NRCS (United States Department of Agriculture/Natural Resources Conservation Service). 1999. Grassland Birds. Available at: http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_009930.pdf. [Std]
- {USDA/NSERL 2004} USDA/NSERL (United States Department of Agriculture/National Soil Erosion Research Laboratory). 2004. Cligen Weather Generator, expanded and improved by USDA Agricultural Research Service and U. S. Forest Service. Available at: <http://horizon.nserl.purdue.edu/Cligen/>. [Std]
- {USGS 2015} USGS (U.S. Geological Survey). 2015. Use Maps for 2012. Available at: http://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php. Accessed on November 21, 2015. [Std]
- {van Hemmen. 1992} van Hemmen JJ. 1992. Agricultural pesticide exposure data bases for risk assessment. Rev. Environ. Contam. Toxicol. 126: 1-85.[Std]
- {van Ravenzwaay and Leibold 2004} van Ravenzwaay B; Leibold E. 2004. A comparison between in vitro rat and human and in vivo rat skin absorption studies. Human and Experimental Toxicology. 23(9):421-30. [Std]
- {Vedula and Yano 1994} Vedula U; Yano BL. 1994. Data Evaluation Record (DER) for unpublished study: NAF-85: Probe and 21-day repeated dose dermal toxicity study in New Zealand White Rabbits. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43701502. 13 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]
- {Vedula et al. 1994} Vedula U; Breslin WJ; Yano BL. 1994. Data Evaluation Record (DER) for unpublished study: XDE-105: Oral gavage teratology study in New Zealand White Rabbits. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43414521. 12 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]
- {Vijayasree et al. 2014} Vijayasree V; Bai H; Mathew TB; George T; Xavier G; Kumar NP; Visalkumar S. 2014. Dissipation Kinetics and Effect of Different Decontamination Techniques on the Residues of Emamectin Benzoate and Spinosad in Cowpea Pods. Environmental Monitoring and Assessment. 186(7):4499-506. [Set01]
- {Villanueva and Walgenbach 2005} Villanueva RT; Walgenbach JF. 2005. Development, Oviposition, and Mortality of *Neoseiulus fallacis* (Acari: Phytoseiidae) in Response to Reduced-Risk Insecticides. Journal of Economic Entomology. 98(6):2114-20. [Set01]
- {Waldbauer 1968} Waldbauer GP. 1968. The consumption and utilization of food by insects. Advances in Insect Physiology. 5: 229-288. [Std]
- {Wang and Messing 2006} Wang XG; Messing RH. 2006. Feeding and Attraction of Non-Target Flies to Spinosad-Based Fruit Fly Bait. Pest Management Science. 62(10):933-9. [Set01]
- {Wang et al. 2012a} Wang DS; He YR; Guo XL; Luo YL. 2012a. Acute Toxicities and Sublethal Effects of Some Conventional Insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology. 105(4):1157-63. [Set01]
- {Wang et al. 2012b} Wang Y; Cang T; Zhao X; Yu R; Chen L; Wu C; Wang Q. 2012b. Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. Ecotoxicology and Environmental Safety. <http://dx.doi.org/10.1016/j.ecoenv.2011.12.016>. [Std-Compendia]
- {Wanner et al. 2002} Wanner KW; Helson BV; Harris BJ. 2002. Laboratory Evaluation of Two Novel Strategies to Control First-Instar Gypsy Moth Larvae with Spinosad Applied to Tree Trunks. Pest Management Science. 58(8):817-24. [Set01]

{Weinberg et al. 1993} Weinberg JT; Kirk HD; Rick DL; Martin MD. 1993. Data Evaluation Record (DER) for unpublished study: Evaluation of the toxicity of SCE-105 to early life stages of the rainbow trout, *Oncorhynchus mykiss* Walbaum. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43414541. 16 pp [DER01]

{Wharton et al. 1965} Wharton DRA; Wharton ML; Lola J. 1965. Blood Volume and Water Content of the Male American Cockroach, *Periplaneta americana* L. – Methods and the Influence of Age and Starvation. Journal of Insect Physiology. 11:391-404. [Std]

{WHO 2008} WHO (World Health Organization). 2008. WHO Specifications and Evaluations for Public Health Pesticides. Spinosad: A mixture of spinosyn A, and spinosyn D with spinosyns A:D proportions in the range 50:50 to 95:5. Document dated March 2008. Available at: http://www.who.int/whopes/quality/Spinosad_eval_only_March_2008.pdf. [Set00]

{WHO 2011} WHO (World Health Organization). 2011. WHO specifications and Evaluations for Public Health Pesticides: Spinosad: A mixture of spinosyn A and spinosyn D, with spinosyns A:D proportions in the range of 50:50 to 95:5. Available from http://www.who.int/whopes/quality/Spinosad_eval_only_Sep_2011.pdf. [Set00]

{Williams and Price 2004} Williams III L; Price LD. 2004. A Space-Efficient Contact Toxicity Bioassay for Minute Hymenoptera, Used to Test the Effects of Novel and Conventional Insecticides on the Egg Parasitoids *Anaphes iole* and *Trichogramma pretiosum*. Biocontrol. 49(2): 163-185. [Set00]

{Williams et al. 2003a} Williams III L; Price LD; Manrique V. 2003. Toxicity of Field-Weathered Insecticide Residues to *Anaphes iole* (Hymenoptera: Mymaridae), an Egg Parasitoid of *Lygus lineolaris* (Heteroptera: Miridae), and Implications for Inundative Biological Control in Cotton. Biological Control. 26(3): 217-223. [Set00]

{Williams et al. 2003b} Williams T; Valle J; Vinuela E. 2003. Is the naturally derived insecticide spinosad compatible with insect natural enemies? Biocontrol Science and Technology. 13(5):459-475. [Set03]

{Willis and McDowell. 1987} Willis, GH; McDowell, LL. 1987. Pesticide persistence on foliage. Reviews of Environmental Contamination and Toxicology. 100: 23-73. [Std]

{Wilmer et al. 1993} Wilmer JW; Spencer PJ; Yano BL; Bond DM. 1993. Data Evaluation Record (DER) for unpublished study: XDE-105: 13-Week neurotoxicity study in Fischer 344 rats. Toxicology Research Lab, Health and Environmental Sciences, The Dow Chemical Co, Midland, MI. MRID No. 43557504. 10 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

{Wimmer et al. 1993} Wimmer MJ; Smith RK; Wallinga DI; Toney SR; Faber DC; Miracle JE; Carnes JT; Rutherford AB. 1993. Persistence of Diflubenzuron on Appalachian Forest Leaves after Aerial Application of Dimilin. J Agric Food Chem. 41: 2184-2190. [Std]

{Winegardner 1996} Winegardner DL. 1996. An Introduction to Soils for Environmental Professionals. CRC Press, Boca Raton, Florida. 270 pp.[Std]

{Winston 1987} Winston ML. 1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, MA. ISBN 0-674-07409-2 280 pp. [Std]

{Wolken et al. 2012} Wolken S; Franc M; Bouhsira E; Wiseman S; Hayes B; Schnitzler B; Jacobs DE. 2012. Evaluation of Spinosad for the Oral Treatment and Control of Flea Infestations on Dogs in Europe. Vet Rec. 170(4):99. [Set01]

{Wright et al. 1992} Wright FL; Keton MJ; Grothe DW. 1992. . Data Evaluation Record (DER) for unpublished study: XDE-105: Acute oral toxicity of XDE-105 administered orally to in Fischer 344 rats. Toxicology Research Lab, Eli Lilly and Co. Indianapolis, IN. MRID No. 43770701. 10 pp. (Taken from U.S. EPA/OPP/HED 1997a). [DER01]

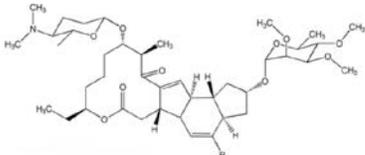
- {Yano et al. 2002} Yano BL; Bond DM; Novilla MN; McFadden LG; Reasor MJ. 2002. Spinosad insecticide: subchronic and chronic toxicity and lack of carcinogenicity in Fischer 344 rats. *Toxicological Sciences*. 65: 288-298. [Set00]
- {York 1993} York J. 1993. Data Evaluation Record (DER) for unpublished study: Acute toxicity of XDE-105 insecticide to the sheepshead minnow (*Cyprindon variegatus*). Environmental Toxicology and Chemistry Research Lab (ESE, Inc.), Dow Chemical CO. Lab Report ID: 392302102003140. MRID No. 43414540. 10 pp. [DER01]
- {Yurk 1993} Yurk J. 1993. Data Evaluation Record (DER) for unpublished study: Acute toxicity of XDE-105 insecticide to the grass shrimp (*Palaemonetes pugio*). Environmental Science and Engineering, Inc., Dow Chemical Co. Lab Report ID: 392302106003140. MRID No. 43414539. 9 pp. [DER01]
- {Zanuncio et al. 2005} Zanuncio JC; Beserra EB; Molina-Rugama AJ; et al. 2005. Reproduction and Longevity of *Supputius cincticeps* (Het.: Pentatomidae) Fed with Larvae of *Zophobas confusa*, *Tenebrio molitor* (Col.: Tenebrionidae) or *Musca domestica* (Dip.: Muscidae). *Brazilian Archives of Biology and Technology*. 48(5): 771-777. [Set00]
- {Zhang et al. 2014} Zhang SK; Ren XB; Wang YC; Su J. 2014. Resistance in *Cnaphalocrocis medinalis* (Lepidoptera: Pyralidae) to New Chemistry Insecticides. *Journal of Economic Entomology*. 107(2):815-20. [Set00]
- {Zhao et al. 2007} Zhao E; Xu Y; Dong M; Jiang S; Zhou Z; Han L. 2007. Dissipation and residues of spinosad in eggplant and soil. *Bulletin of Environmental Contamination and Toxicology*. 78:222–225. (As cited in Mandal et al. 2013). [Set02]

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-Marín 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. <i>Several additional studies</i>
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 GENECC2 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

Item	Value	Reference ^[1]												
	Buffered water <table border="1" data-bbox="467 260 1045 417"> <thead> <tr> <th data-bbox="467 260 662 317">pH</th> <th data-bbox="662 260 857 317">Spinosyn A (mg/L)</th> <th data-bbox="857 260 1045 317">Spinosyn D (mg/L)</th> </tr> </thead> <tbody> <tr> <td data-bbox="467 317 662 352">5</td> <td data-bbox="662 317 857 352">290</td> <td data-bbox="857 317 1045 352">29</td> </tr> <tr> <td data-bbox="467 352 662 388">7</td> <td data-bbox="662 352 857 388">235</td> <td data-bbox="857 352 1045 388">0.332</td> </tr> <tr> <td data-bbox="467 388 662 424">9</td> <td data-bbox="662 388 857 424">16</td> <td data-bbox="857 388 1045 424">0.053</td> </tr> </tbody> </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.	pH	Spinosyn A (mg/L)	Spinosyn D (mg/L)	5	290	29	7	235	0.332	9	16	0.053	Dow Elanco 1996; Thompson et al. 2005 U.S. EPA/OPP/EFED 2011a U.S. EPA/OPP/HED 2009a
pH	Spinosyn A (mg/L)	Spinosyn D (mg/L)												
5	290	29												
7	235	0.332												
9	16	0.053												
Environmental Properties														
Aquatic anaerobic metabolism, half-lives	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.	MRID 43507305, U.S. EPA/OPP/EFED 2005, 2009a,b Also in Tomlin 2004												
	Spinosyn A: 160 days Spinosyn D: 240 days	Cleveland et al. 2002a												
Aqueous photolysis, half-lives	≈1 day, pH 7, 25°C	Dow Elanco 1996 Cleveland et al. 2002a												
Bioconcentration in fish (BCF, L/kg)	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)	MRID 43557601, U.S. EPA/OPP/EFED 2005, 2009a												
	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)	MRID 44537734, U.S. EPA/OPP/EFED 2005, 2009a												
	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)	U.S. EPA/OPP/EFED 2009a, p. 8 MRID not specified.												
Field dissipation	Less than 2-3 weeks	Dow Elanco 1996												
Foliar half-life	1.6-16 days	Tomlin 2004												
	35 days used as default ...to account for the stability of spinosad (EFED p. 46).	U.S. EPA/OPP/EFED 2011a												
	Zucchini: 3.6 to 4.1 days [3.5, 3.6, 3.9, 3.9; Table 2 of paper]	Liu et al. 2013b												
	Cauliflower: 1.2 days at 15 g/ha 1.58 days at 30 g/ha Average: 1.4 days	Mandal et al. 2009												
	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.	Santis et al. 2012												

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

Item	Value	Reference ^[1]									
Sediment half-life	Spinosyn A: 161 days Spinosyn D: 250 days Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENECC2 in EFED 2009b drinking water assessment.	U.S. EPA/OPP/EFED 2009b									
Soil half-life, aerobic	Spinosyn A: 9.4-17.3 days	Dow Elanco 1996									
	Spinosyn A: 28 (volcanic soil) Spinosyn D: 37 (volcanic soil)	Cleveland et al. 2002a									
	Spinosyn A: 9 days (sandy loam), 17 days (silt loam) Spinosyn D: 14 days (silt loam) Much longer half-lives sterilized soils.	Hale and Portwood 1996									
	Spinosyn A: 9.4-17.3 days Spinosyn D: 14.5 days	Tomlin 2004									
	<table border="1"> <thead> <tr> <th>Soil</th> <th>Spinosyn A (t_{1/2} days)</th> <th>Spinosyn D (t_{1/2} days)</th> </tr> </thead> <tbody> <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> </tbody> </table> Working Note: Spinosad assumed to be stable for PRZM/EXAMS and GENECC2 in EFED 2009a,b drinking water assessment.	Soil	Spinosyn A (t _{1/2} days)	Spinosyn D (t _{1/2} days)	Silt loam	17.3	14.5	Sandy loam	9.4		MRID 43507304, U.S. EPA/OPP/EFED 2005, 2009a Working Note: Identical to Hale and Portwood 1996 and Tomlin 2004.
Soil	Spinosyn A (t _{1/2} days)	Spinosyn D (t _{1/2} days)									
Silt loam	17.3	14.5									
Sandy loam	9.4										
Soil dissipation half-life	Spinosyn A Silt loam: 0.5 days (Mississippi) Loam: 0.3 days (California)	MRID 43714301, U.S. EPA/OPP/EFED 2005, 2009a									
	3.5 to 3.9 days [3.8, 4.1, 4.0, 3.6: Table 2 of paper]	Liu et al. 2013b									
	2.8 days (at 17.5 g/ha) 2.0 days (at 35 g/ha)	Sharma et al. 2007									
	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.	Thompson et al. 2002a,b									
	Spinosyn A: 1.87 days Spinosyn D: 0.95 days	Zhao et al. 2007									
Water Dissipation	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	MRID 43848803, U.S. EPA/OPP/EFED 2005, 2009a									
	Outdoor microcosm half-life: 1.8 days (Spinosyns A and D)	Cleveland et al. 2002a.									

^[1] There are many sources of information on some standard values – e.g., molecular weight. In general, only two sources are 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 Labeled 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. Aerial: 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%)	Aerial: 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. Aerial: 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. Aerial: 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.msdsolnline.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 LC₅₀ 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]		
<i>Daphnia</i> Acute LC ₅₀	1.5	1.5	1.5 ^[6]	1.5 ^[6]		
<i>Daphnia</i> 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 , <i>A. flos-aquae</i>		6.1				
Algae EC ₅₀ , <i>Navicula</i>	0.107	0.107	0.107 ^[6]	0.107 ^[6]		
Algae EC ₅₀ , <i>P. subcapitata</i>	39	39		39 ^[6]		
<i>Lemna</i> 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 10th.

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) [$k_{a_{Ref}}$]	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) [k_{a_p}]	0.00002	Section 3.1.3.2.2	9
$k_{a_p} \div k_{a_{Ref}}$	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) [$k_{a_{Ref}}$]	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) [k_{a_p}]	0.00002	Section 3.1.3.2.2	9
$k_{ap} \div k_{a_{Ref}}$	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) [$k_{a_{Ref}}$]	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) [k_{a_p}]	0.00002	Section 3.1.3.2.2	9
$k_{a_p} \div k_{a_{Ref}}$	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_{oc} , 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. $WCR = 2.15 \mu\text{g/L} \div (0.094 \times 5) \approx 4.5745 \mu\text{g/L per lb a.i./acre}$. 60-day concentration of 1.12 µg/L for 5 applications at an application rate of 0.094 lb a.i./acre. $WCR = 1.12 \mu\text{g/L} \div (0.094 \times 5) \approx 2.383 \mu\text{g/L per lb a.i./acre}$.

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 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 \times (Central Value \div 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 LD₅₀ 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]						
<i>Apis mellifera</i>	48	TGAI	47.11	116	0.41	Carvalho et al. 2013
<i>Apis mellifera</i>	N.S.	TGAI	57	116	0.49	European Commission 2006
<i>Apis mellifera</i>	48	TGAI	63	128	0.492	Mayer et al. 2001
<i>Apis mellifera</i>	24	TGAI	60	116	0.52	Miles et al. 2002
<i>Apis mellifera</i>	48	TGAI	53	116	0.50	Mayes et al. 2003
<i>Apis mellifera</i>	N.S.	NAF-85	49	116	0.42	European Commission 2006
<i>Apis mellifera</i>	48	480 SC	53	116	0.50	Mayes et al. 2003
<i>Bombus terrestris</i>	48	480 SC	38.5	300	0.13	Mayes et al. 2003
<i>Melipona quadrifasciata</i>	24	480 SC	12.07	80	0.15	Tom et al. 2015
Blattodea: Blattidae						
<i>Periplaneta americana</i> ^[3]	24	Spyn A	740	700	1.1	Salgado 1998
<i>Periplaneta americana</i> ^[3]	24	Spyn A	1900	700	2.7	Salgado 1998
Lepidoptera						
<i>Heliothis virescens</i> (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 LD₅₀ 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
<i>Aedes aegypti</i>	2.85	Pridgeon et al. 2008, Table 2
<i>Anopheles quadrimaculatus</i>	1.92	Pridgeon et al. 2008, Table 4
<i>Apis mellifera</i>	116	Winston (1987, p. 54)
<i>Bombus impatiens</i>	150	Franklin et al. (2004)
<i>Bombus terrestris</i>	300	Thompson 2015, Table 1, p. 2
<i>Bactrocera dorsalis</i>	15	Lin et al. 2013, Table 2, p. 356
<i>Bactrocera cucurbitae</i>	15	Use value for <i>Bactrocera dorsalis</i>
<i>Culex quinquefasciatus</i>	2.02	Pridgeon et al. 2008, Table 3
<i>Helicoverpa armigeram</i>	11.5	Hertog et al. 2002, Table 5, average of range 8-15 mg.
<i>Heliothis virescens</i> (larva)	60	Salgado 1998, p. 95, 50 to 70 mg
<i>Hyposoter didymator</i> (pupa)	19.8	Schneider et al. 2003
<i>Manduca sexta</i>	30	Hertog et al. 2002, average of 20 to 40 mg range
<i>Musca domestica</i>	30	Zanuncio et al. 2005, Figure 1a, p. 774
<i>Megachile rotundata</i>	30	Thompson 2015, Table 1, p. 2 and Meyer et al. 2001
<i>Melipona quadrifasciata</i>	80	Thompson 2015, Table 1, p. 2, average of 2 other <i>Melipona</i> species.
<i>Nomia melander</i>	85	Mayer et al. 2001
<i>Periplaneta americana</i>	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 LD₅₀ 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]						
<i>Apis mellifera</i>	48	TGAI	2.9	116	0.025	Hoxter et al. 1992 ^[2]
<i>Apis mellifera</i>	N.S.	N.S.	3.6	116	0.031	European Commission 2006
<i>Apis mellifera</i>	48	TGAI	40	116	0.34	Mayes et al. 2003 ^[5]
<i>Apis mellifera</i>	48	TGAI	47.11	116	0.41	Carvalho et al. 2013
<i>Apis mellifera</i>	24	1.6% WP	50	116	0.43	Miles et al. 2002
<i>Apis mellifera</i> ^[4]	24	TGAI	78	127	0.61	Mayer et al. 2001
<i>Apis mellifera</i>	48	480 SC	60	116	0.52	Mayes et al. 2003 ^[5]
<i>Apis mellifera</i>	24	480 SC	880	116	7.6	Miles 2003
<i>Apis mellifera</i>	48	480 SC	900	116	8.5	Mayes et al. 2003 ^[5]
<i>Megachile rotundata</i> [Megachilidae] ^[4]	24	TGAI	58	30	1.9	Mayer et al. 2001
<i>Nomia melander</i> [Halictidae] ^[4]	24	TGAI	65	85	0.76	Mayer et al. 2001
<i>Bombus terrestris</i>	48	480 SC	19,400	300	65	Mayes et al. 2003 ^[5,6]
Hymenoptera: Ichneumonidae						
<i>Hyposoter didymator</i> [p]	48	SC	N.S.	19.8	0.5	Schneider et al. 2003
Diptera: Muscidae						
<i>Musca domestica</i>	72	N.S.	24.2	30	0.8	Scott 1998
<i>Musca domestica</i>	72	TGAI	0.74	30	0.025	Shi et al. 2011
Diptera: Culicidae						
<i>Aedes aegypti</i>	24	N.S.	N.S.	2.85	0.89	Pridgeon et al. 2008
<i>Culex quinquefasciatus</i>	24	N.S.	N.S.	2.02	2.02	Pridgeon et al. 2008
<i>Anopheles quadrimaculatus</i>	24	N.S.	N.S.	1.92	1.5	Pridgeon et al. 2008
Diptera: Tephritidae fruit fly						
<i>Bactrocera dorsalis</i>	24	TGAI	59.6	15	3.97	Hsu and Fend 2006
<i>Bactrocera dorsalis</i>	24	TGAI	40.9	15	2.73	Hsu et al. 2012b
<i>Bactrocera cucurbitae</i>	24	SC	5.0	15*	0.33	Hsu et al. 2012a
Lepidoptera: Sphingidae						
<i>Manduca sexta</i> (larvae)	48	N.S.	2.0	30	0.067	Herzog et al. 2002
Lepidoptera: Noctuidae						
<i>Helicoverpa armigeram</i>	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 LD₅₀ of 2.5 µg/bee is reported in review by Miles (2003). This is probably identical to Hoxter study.

^[3] Reported LD₅₀ 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 1999a. 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 LC₅₀ (mg/L)	Confidence Interval on LC₅₀ (mg/L)	Sensitivity Relative to the Honeybee ^[1]	Reference
Honey bee, <i>Apis mellifera</i> [Apidae]	22	18-25	1	Bailey et al. 2005
Alfalfa leafcutting bee, <i>Megachile rotundata</i> [Megachilidae]	12.5	11.3-14	0.56	Scott-Dupree et al. 2009
Bumblebee, <i>Bombus impatiens</i> [Apidae]	89.5	79.2-100.6	4.07	Scott-Dupree et al. 2009
Blue orchard bee, <i>Osmia lignaria</i> [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	<i>Zygogramma exclamationis</i>	Sunflower Beetle	0.045	0.13
Coleoptera	Chrysomelidae	<i>Leptinotarsa decemlineata</i>	Colorado Potato Beetle	0.045	0.38
Coleoptera	Chrysomelidae	<i>Epitrix fuscula</i>	Eggplant Flea Beetle	0.045	0.63
Coleoptera	Chrysomelidae	<i>Leptinotarsa decemlineata</i>	Colorado Potato Beetle	0.053	0.88
Diptera	Agromyzidae	<i>Liriomyza trifolii</i>	Serpentine Leafminer	0.090	0.50
Hemiptera	Anthocoridae	<i>Orius insidiosus</i>	Minute Pirate Bug	0.089	0.17
Hemiptera	Anthocoridae	<i>Orius insidiosus</i>	Minute Pirate Bug	0.090	0.50
Hemiptera	Anthocoridae	<i>Orius insidiosus</i>	Minute Pirate Bug	0.199	0.83
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.016	0.02
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.025	0.05
Lepidoptera	Noctuidae	<i>Trichoplusia ni</i>	Cabbage Looper	0.026	0.09
Lepidoptera	Pieridae	<i>Pieris rapae</i>	Cabbage White	0.026	0.13
Lepidoptera	Plutellidae	<i>Plutella xylostella</i>	Diamondback Moth	0.026	0.16
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.030	0.20
Lepidoptera	Noctuidae	<i>Anticarsia gemmatalis</i>	Velvetbean Caterpillar	0.030	0.23
Lepidoptera	Noctuidae	<i>Trichoplusia ni</i>	Cabbage Looper	0.045	0.27
Lepidoptera	Pieridae	<i>Pieris rapae</i>	Cabbage White	0.045	0.30
Lepidoptera	Plutellidae	<i>Plutella xylostella</i>	Diamondback Moth	0.045	0.34
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.045	0.38
Lepidoptera	Noctuidae	<i>Helicoverpa zea</i>	Corn Earworm	0.045	0.41
Lepidoptera	Sphingidae	<i>Manduca sexta</i>	Hawk Moth	0.045	0.45
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.045	0.48
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.050	0.52
Lepidoptera	Sphingidae	<i>Manduca sexta</i>	Hawk Moth	0.050	0.55
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.050	0.59
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.060	0.63
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.060	0.66
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.067	0.70
Lepidoptera	Noctuidae	<i>Chrysodeixis includens</i>	Soybean Looper Moth	0.067	0.73
Lepidoptera	Crambidae	<i>Ostrinia nubilalis</i>	European Corn Borer	0.068	0.77
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.073	0.80
Lepidoptera	Gelechiidae	<i>Anarsia lineatella</i>	Peach Twig Borer	0.094	0.84
Lepidoptera	Tortricidae	<i>Rhyacionia frustrana</i>	Nantucket Pinetip Moth	0.098	0.88
Lepidoptera	Noctuidae	<i>Spodoptera frugiperda</i>	Fall Armyworm	0.100	0.91
Lepidoptera	Noctuidae	<i>Spodoptera exigua</i>	Beet Armyworm	0.101	0.95
Lepidoptera	Noctuidae	<i>Agrotis ipsilon</i>	Cutworm	0.269	0.98
Orthoptera	Acrididae	<i>Melanoplus femurrubrum</i>	Redlegged Grasshopper	0.090	0.50
Hymenoptera	Pteromalidae	<i>Catolaccus grandis</i>	Ectoparasitoid Wasp	0.022	0.10
Hymenoptera	Chalcidoidea	<i>Trichogramma exiguum</i>	Parasitic Wasp	0.070	0.30
Hymenoptera	Eurytomidae	<i>Eurytoma pini</i>	Chalcid Wasp	0.098	0.50
Hymenoptera	Chalcididae	<i>Haltichella rhyacioniae</i>	Chalcid Wasp	0.098	0.70
Hymenoptera	Braconidae	<i>Macrocentrus ancyliivorus</i>	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				
	<i>Cryptolestes ferrugineus</i>	1.7	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
	<i>Rhyzopertha dominica</i>	0.6-1.0	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
	<i>Tribolium castaneum</i>	4.8-7.5	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
Diptera				
	<i>Bactrocera cucurbitae</i>	≈10 to 13.3	Field populations. No additional selection pressure.	Hsu et al. 2012a
	<i>Bactrocera dorsalis</i>	>480	Eight generations of resistance pressure based on survival of bioassays at LD₇₀. No marked cross-resistance to other pesticides.	Hsu and Feng 2006
	<i>Bactrocera dorsalis</i>	>2445	30 generations of selection pressure. Mechanism unclear but possibly related to changes in receptor.	Hsu et al. 2012b
	<i>Musca domestica</i>	>150	Field population. No additional resistance pressure. Resistance apparently due to altered target site	Gao et al. 2007a
	<i>Drosophila melanogaster</i>	3.8 to 7408	Various resistant and sensitive strains. No selection pressure during study.	Rinkevich and Scott 2013
	<i>Musca domestica</i>	21	Selection pressure (variable dosing) over 22 generations. Potential partial involvement of P450.	Markussen and Kristensen 2012
	<i>Musca domestica</i>	0.9 to 4.3	Strains with known resistance to other pesticides. No additional selection pressure.	Scott 1998
	<i>Musca domestica</i>	279	Selection pressure (60-80% lethal doses) over 27 generations. No apparent involvement of P450.	Shi et al. 2011
Lepidoptera				
	<i>Cnaphalocrocis medinalis</i>	0.39-3	Field populations subject to three years of spinosad field use. No other substantial selection pressure.	Zhang et al. 2014
	<i>Helicoverpa armigera</i>	0.5 to 1.6	No cross resistance to spinosad in a strain resistant to pyrethroids.	Achaleke et al. 2009
	<i>Plodia interpunctella</i>	1.75	Field versus laboratory populations. No additional resistance pressure. Variability rather than resistance?	Huang et al. 2004
	<i>Plutella xylostella</i>	1983	Selection pressure for 11 generation. Resistance associated with increased P450 activity.	Sayyed et al. 2008
	<i>Tuta absoluta</i>	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				
	<i>Frankliniella occidentalis</i>	3,682 to 2,968,500	15 wild caught strains in areas with spinosad use. Resistance pressure for at least 7 generations (LD_{50S}). 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
	<i>Frankliniella occidentalis</i>	20,000 to >100,000	Resistance pressure based on survivors of LD₅₀ exposures over 4 generations. Resistance stable for up to 8 months in the absence of breeding with sensitive strains.	Bielza et al. 2008
	<i>Frankliniella occidentalis</i>	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
	<i>Thrips palmi</i>	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				
<i>Daphnia magna</i>	TGAI	48	7.37	Cleveland et al. 2002b
<i>Daphnia magna</i>	TGAI	48	14	Milazzo et al. 1994, MRID 43574502
<i>Daphnia magna</i>	TGAI	48	9.1	European Commission 2006
<i>Daphnia magna</i>	Success® formulation	48	0.0048	Deardorff and Stark 2009
<i>Daphnia pulex</i>	Success® formulation	48	0.129	Deardorff and Stark 2009
<i>Ceriodaphnia dubia</i>	Success® formulation	48	0.0018	Deardorff and Stark 2009
Mosquito Larvae				
<i>Aedes aegypti</i>	TGAI	24	0.35	Darriet et al. 2005
<i>Aedes aegypti</i> , 3rd instar	Laser®	48	0.007	Romi et al. 2006
<i>Aedes aegypti</i> , 4th instar	Tracer® Naturalyte	1	0.026	Perez et al. 2007
<i>Aedes aegypti</i>	N.S.	24	51.7	Kovendan et al. 2012
<i>Aedes albopictus</i> , 4th instar	TGAI, 88%	24	0.3	Liu et al. 2004b
<i>Aedes albopictus</i> , 4th Instar	Tracer® 24SC	48	0.019	Khan et al. 2011
<i>Anopheles gambiae</i>	TGAI	24	0.01	Darriet et al. 2005
<i>Anopheles stephensi</i> , 1st instar	NOS	24	0.002	Kumar et al. 2011
<i>Anopheles stephensi</i> , 3rd instar	Laser®	48	0.024	Romi et al. 2006
<i>Culex pipiens</i> , 3 rd instar	Laser®	48	0.0032	Romi et al. 2006
<i>Culex pipiens</i> , 3 rd instar	Conserve®	24	0.027	Cetin et al. 2005
<i>Culex pipiens</i> , 4th instar	Tracer® 12% SC	24	0.087	Mansour et al. 2012
<i>Culex quinquefasciatus</i>	TGAI	24	0.093	Darriet et al. 2005
<i>Culex quinquefasciatus</i> , 2 nd instar	TGAI	48	0.019	Jiang and Mulla 2009
<i>Culex quinquefasciatus</i> , 4 th instar	TGAI	48	0.026	Jiang and Mulla 2009
<i>Culex quinquefasciatus</i> , 4 th instar	TGAI, 88%	24	0.1	Liu et al. 2004a
<i>Culex quinquefasciatus</i> , 3 rd instar	Natular® XRG	24	0.196 to 0.490	Su and Chen 2014b
<i>Culex quinquefasciatus</i> , 2 nd instar	11.6% formulation	48	0.01	Jiang and Mulla 2009
<i>Culex quinquefasciatus</i> , 4th instar	11.6% a.i. formulation	48	0.013	Jiang and Mulla 2009
<i>Culex quinquefasciatus</i> , 3rd instar	Natular® 2EC	72	0.031	Jones and Ottea 2013
Other				
<i>Chironomus circumdatus</i> (midge)	NOS	24	0.009	Kumar et al. 2011
<i>Crassostrea virginica</i> (bivalve)	TGAI	96	0.3	MRID 43571203
<i>Palaemonetes pugio</i> (shrimp)	TGAI	96	>9.76	MRID 43414539
<i>Leptocheirus plumulosus</i> (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]					
<i>Daphnia magna</i>	TGAI, 88%	21	0.62	1.2	MRID 43848801 ^[1,2]
<i>Daphnia magna</i>	NOS	21	1.2	N.S.	European Commission 2006 ^[2]
<i>Daphnia magna</i>	NOS	21	8	N.S.	European Commission 2006 ^[3]
<i>Daphnia magna</i>	Conserve [®] 120SC	14	N.D.	8	Duchet et al. 2010b
<i>Daphnia pulex</i>	Conserve [®] 120SC	14	N.D.	8	Duchet et al. 2010b
<i>Daphnia pulex</i>	Success [®] formulation	60 ^[4]	N.D.	2	Stark and Vargas 2003
<i>Daphnia pulex</i>	NOS	10		129 ^[5]	Stark 2005
<i>Ceriodaphnia dubia</i>	Success [®] formulation	8	0.5	1	Deardorff and Stark 2011
Midges [Diptera: Chironomidae]					
<i>Chironomus riparius</i>	TGAI	25	0.622	1.328	MRID 44828402 ^[1]
<i>Chironomus riparius</i>	NOS	25	1.6	3.2	Cleveland et al. 2002a
<i>Chironomus riparius</i>	NOS	25	1.6	N.S.	European Commission 2006
Shrimp [Mysida: Mysidae]					
<i>Mysidopsis bahia</i>	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]	<i>Apis mellifera</i>	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.

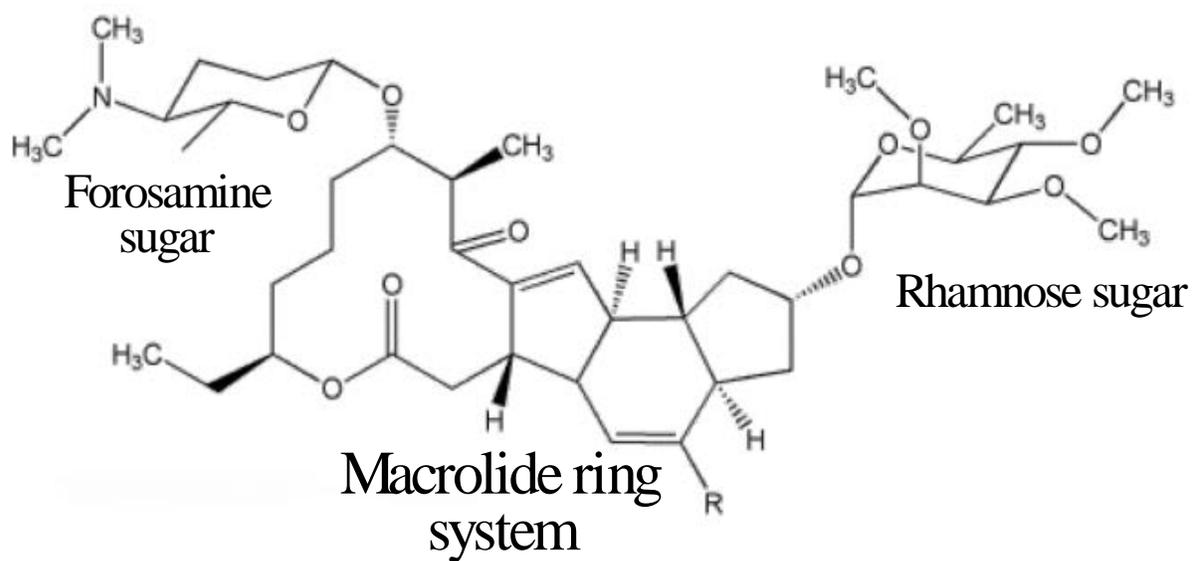
See Section 4.2.3.4 for discussion

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	<i>Ceriodaphnia</i> NOAEC	0.0005 mg/L	Section 4.3.3.3
	Tolerant	Midge NOAEC	0.0842 mg/L	
Aquatic Plants				
Algae	Sensitive	<i>N. pelliculosa</i> , NOAEC	0.05 mg/L	Section 4.3.3.4
	Tolerant	<i>S. capricornutum</i> , NOAEC	4.3 mg/L	Section 4.3.3.4
Macrophytes	Sensitive	No identified		Section 4.3.3.4
	Tolerant	<i>Lemna</i> , NOAEC	1.86 mg/L	Section 4.3.3.4

^[1] This is the NOAEL from the study reporting the lowest topical of LD₅₀ of 0.0029 µg a.i./bee (Hoxter et al. 1992). Other toxicity studies in honeybees report substantially higher topical LD₅₀ 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.



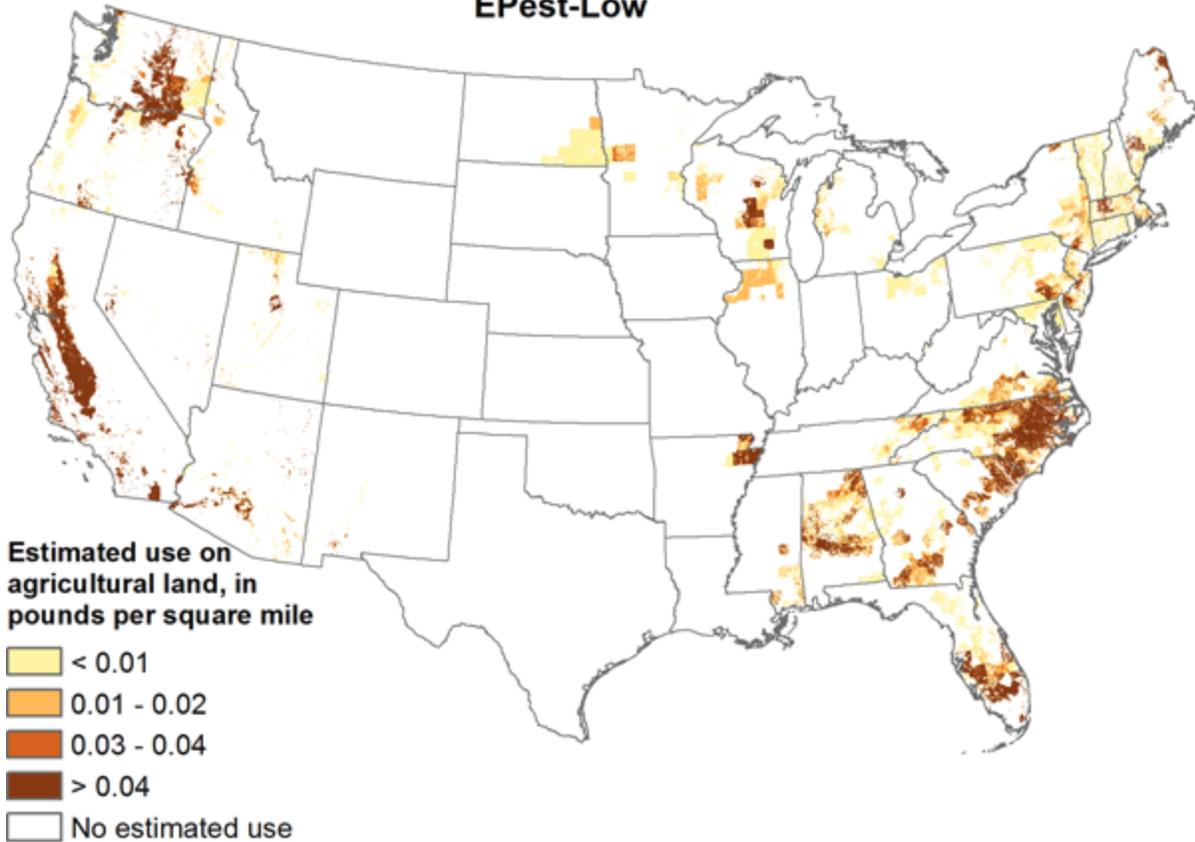
Spinosyn A (R=H) Spinosyn D (R=CH₃)

Figure 1: Structures of Spinosyn A and D

Source: Modified from EFSA 2011, p. 7

Estimated Agricultural Use for Spinosyn, 2012

EPEst-Low



Use by Year and Crop

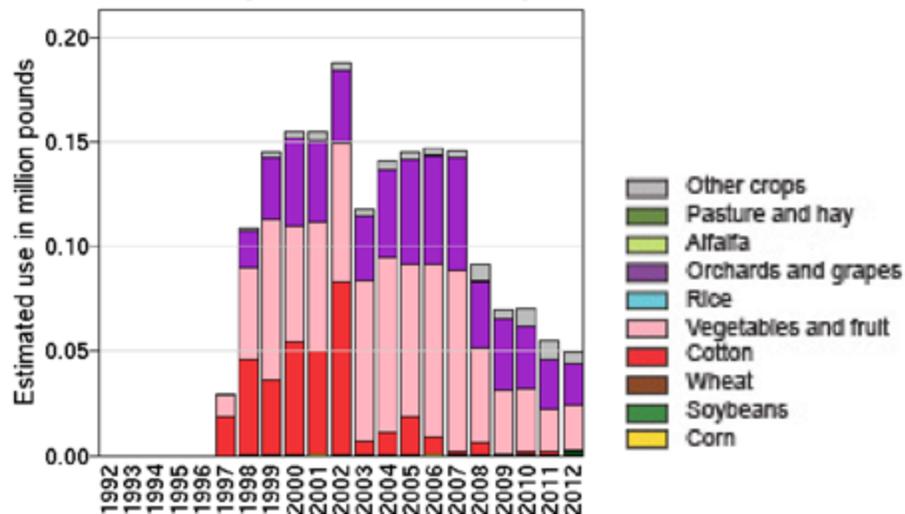


Figure 2: Lower Bound Estimated Agricultural Use of "Spinosyn" for 2012

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

Estimated Agricultural Use for Spinosyn, 2012 EPest-High

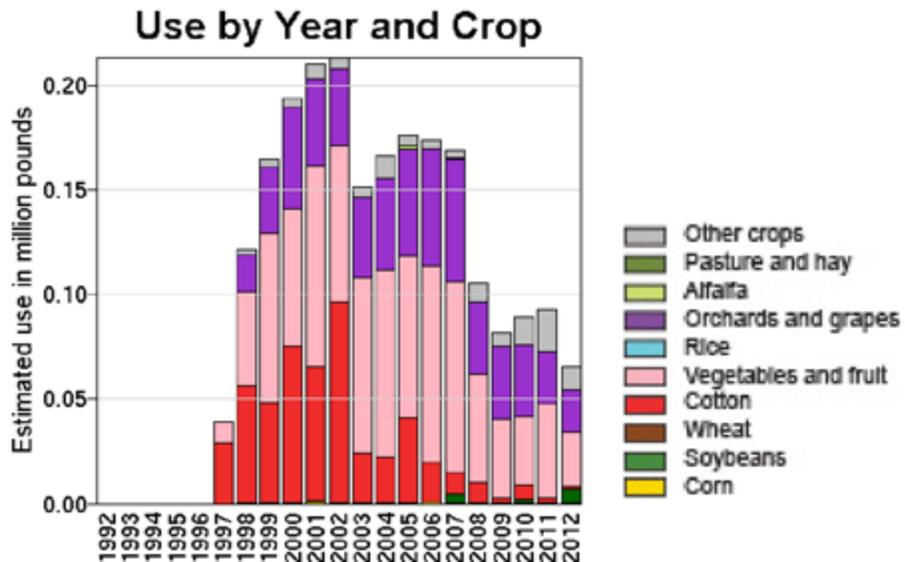
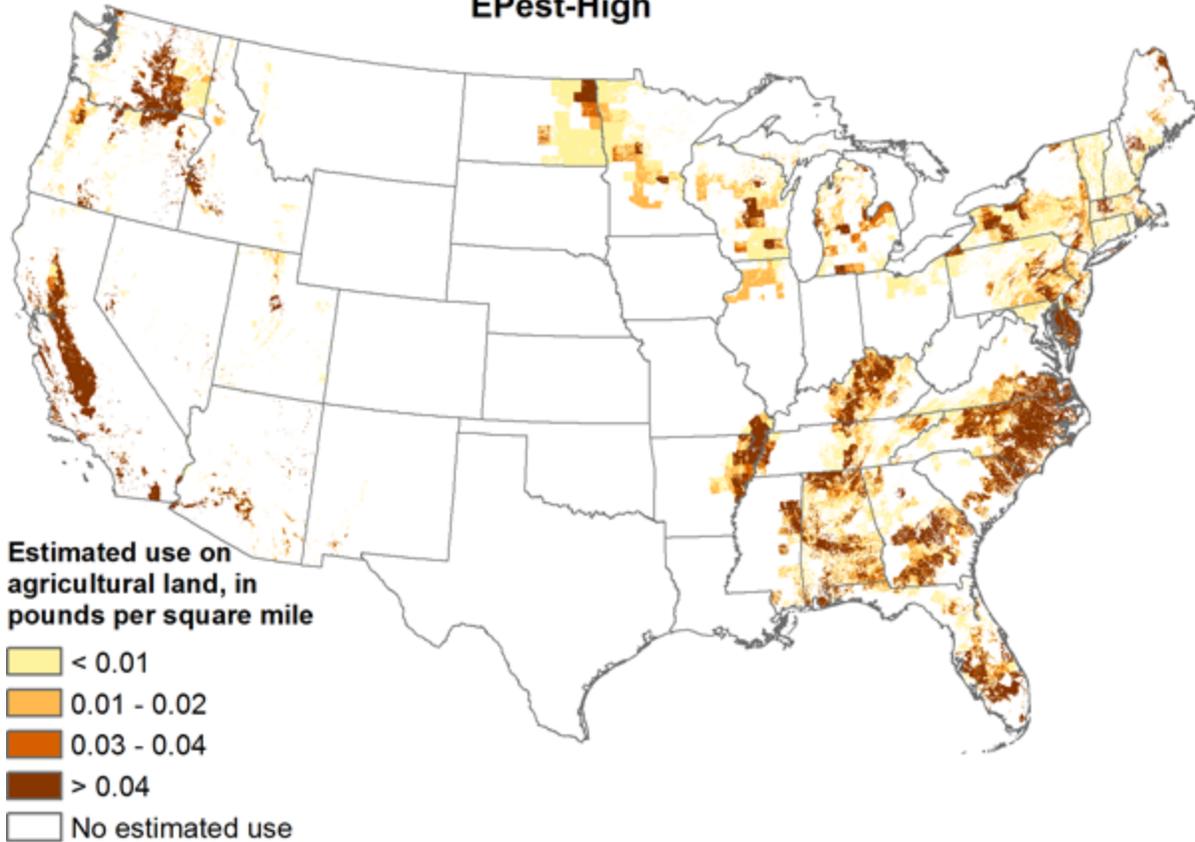


Figure 3: Upper Bound Estimated Agricultural Use of “Spinosyn” for 2012

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

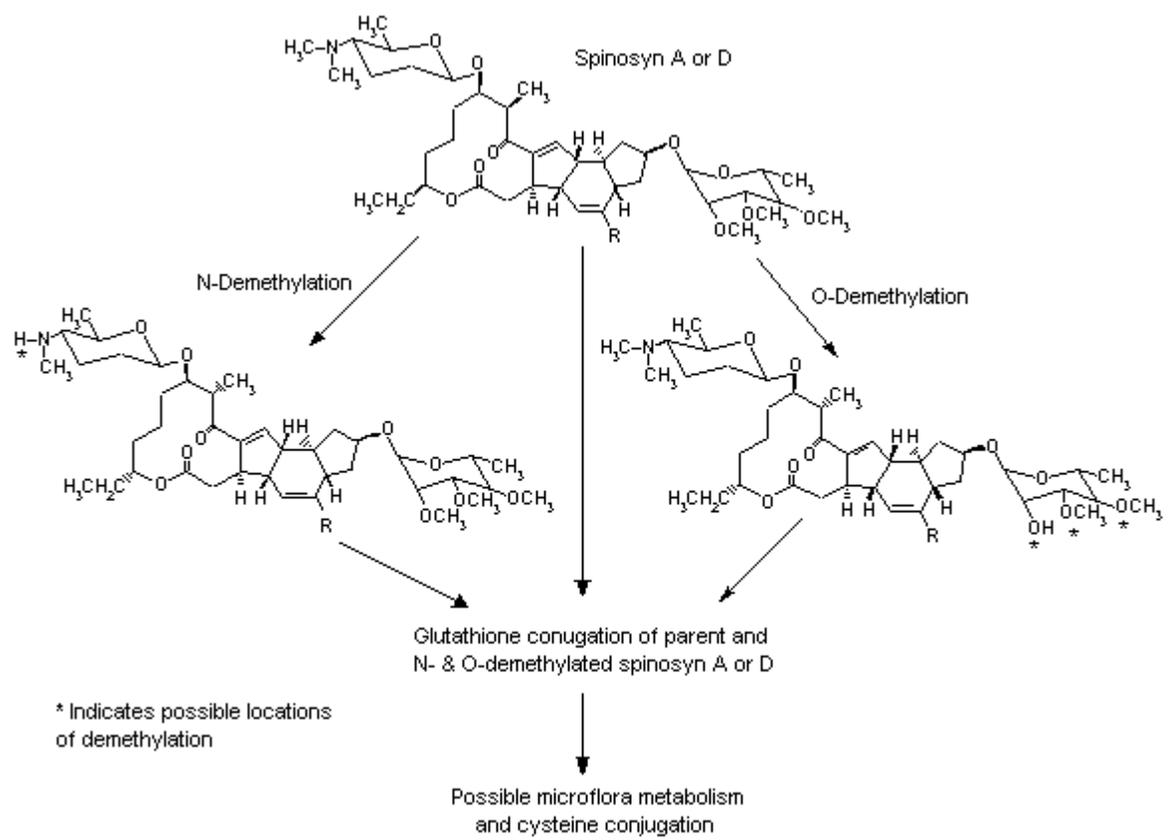


Figure 4: Metabolic pathway in mammals for spinosad

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

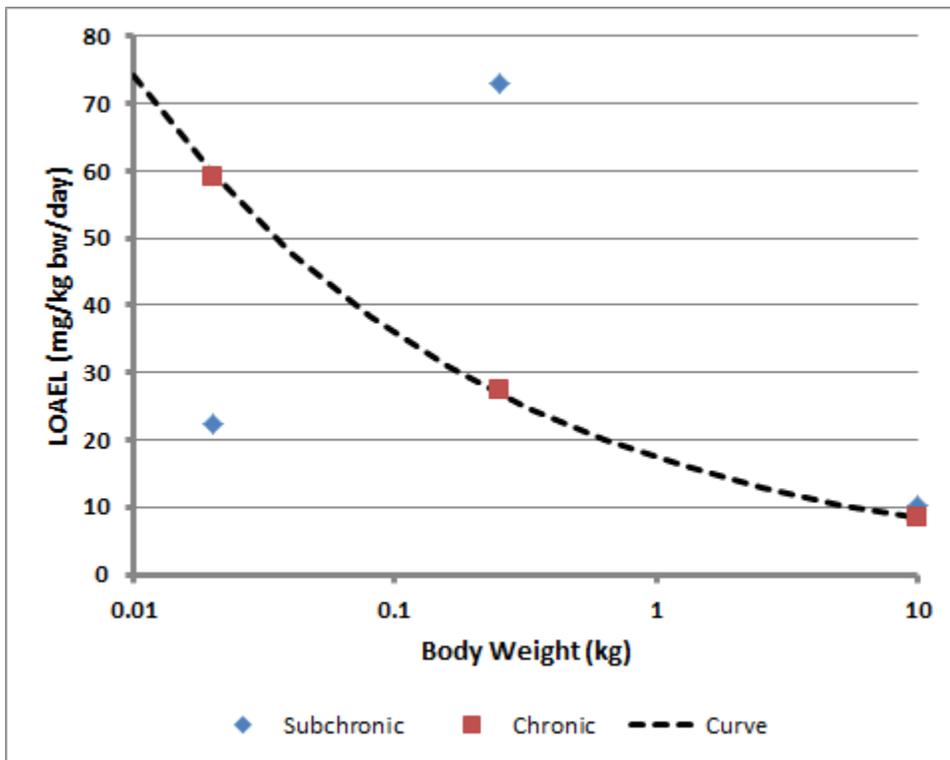


Figure 5: Mammalian Sensitivities to Spinosad

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

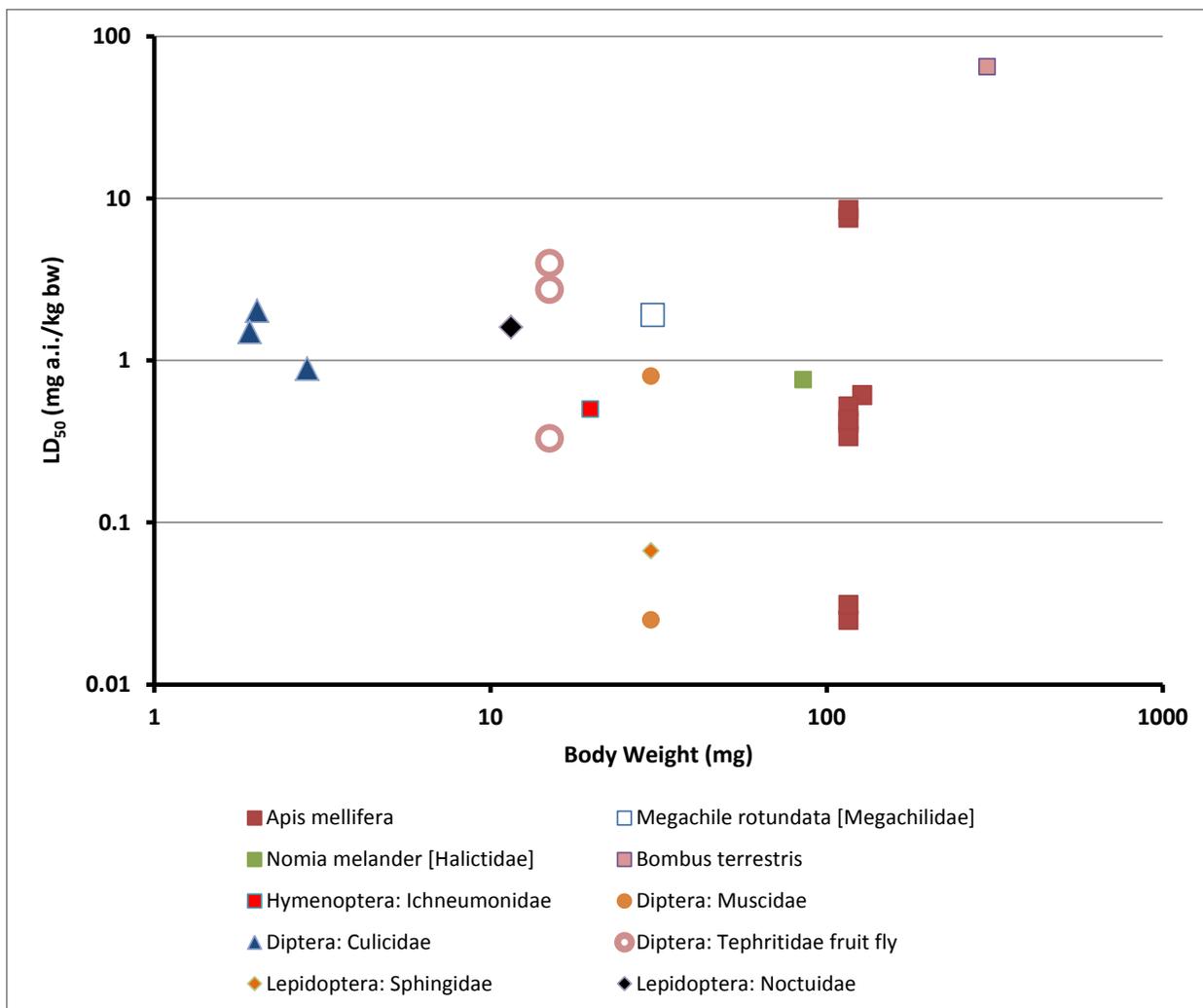


Figure 6: Topical LD₅₀ Values in Insects by Body Weight

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

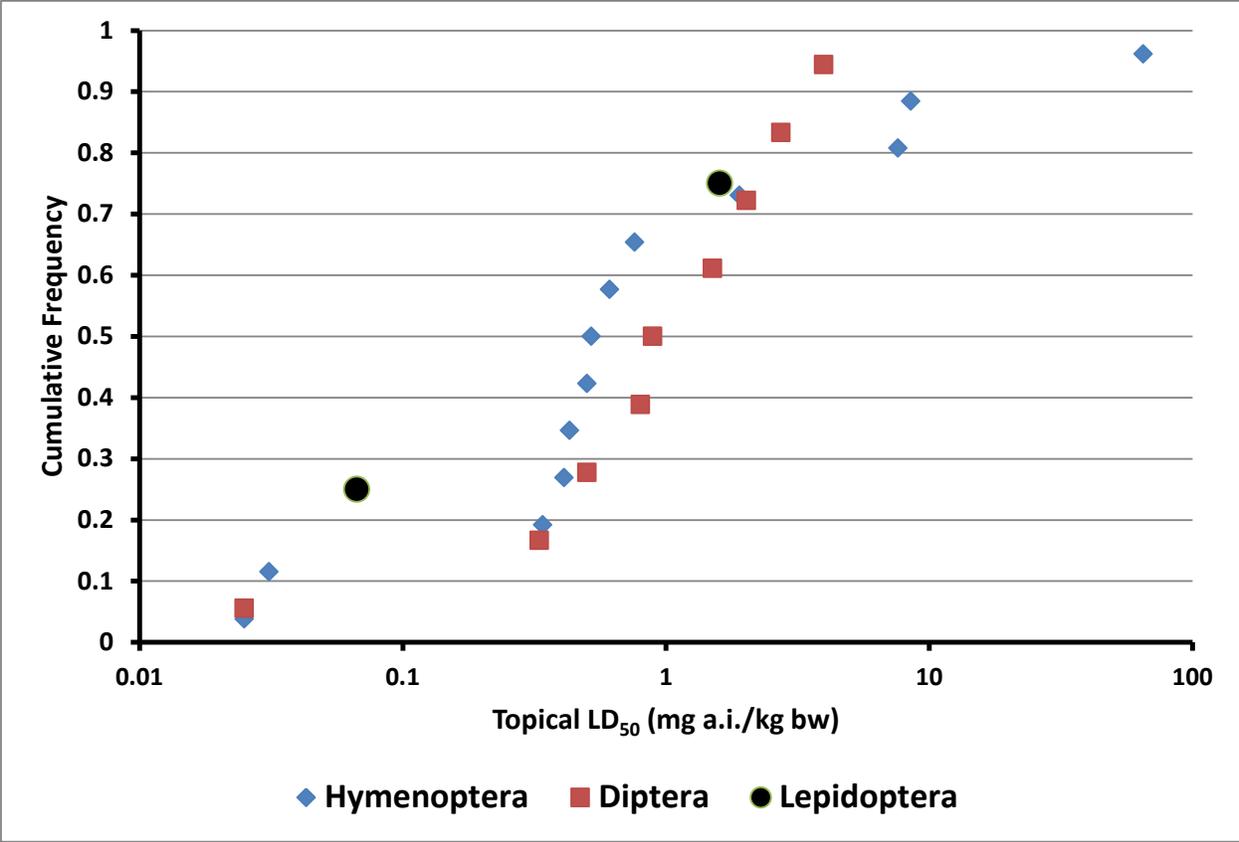


Figure 7: Sensitivity Distributions by Insect Order of Contact LD₅₀ Values

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

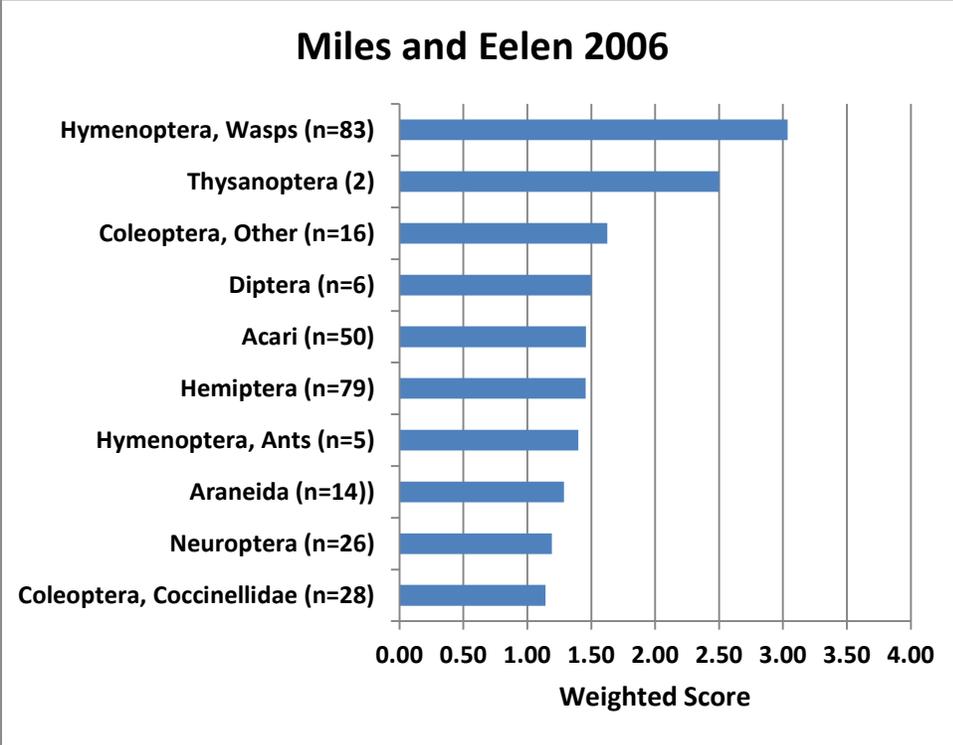
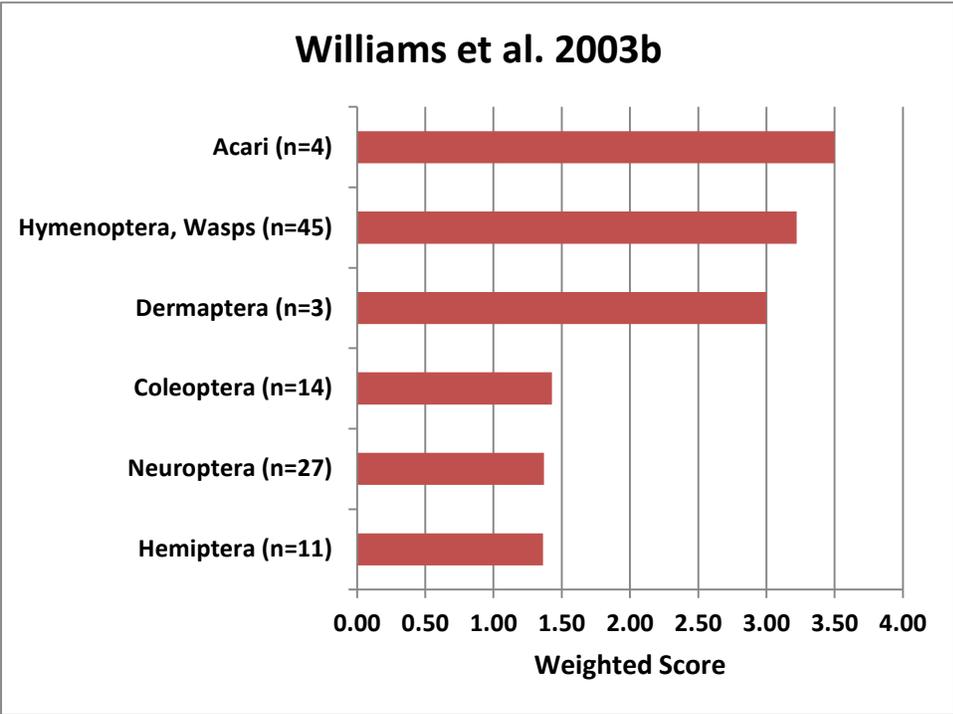


Figure 8: Weighted IOBC Scores for Spinosad

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

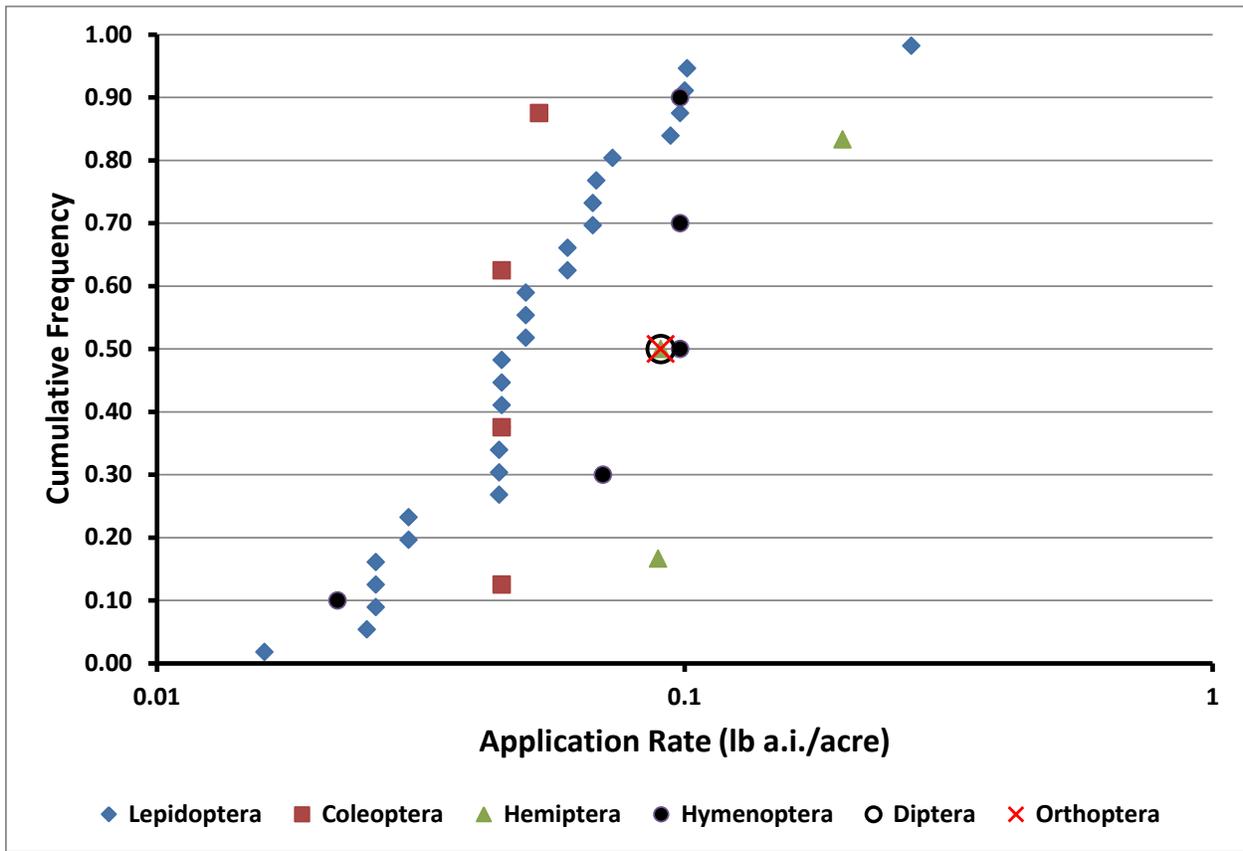


Figure 9: LOAECs from Field Studies for Orders of Terrestrial Insects

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

Appendix 1: Toxicity to mammals.

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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 LD₅₀ 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 males. 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.

Appendix 1: Toxicity to mammals (*continued*)

Table A1-2: Subchronic and Chronic Oral Toxicity Studies

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Subchronic			
Dogs, beagle	<p>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.</p>	<p>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</p>	<p>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.</p>
Mice, CD-1 strain	<p>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.</p>	<p>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).</p>	<p>MRID 43566602 U.S. EPA/OPP/HED 1997b, 2009a</p>

Appendix 1: Toxicity to mammals (*continued*)

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Mice, CD-1 strain, 10 per sex per dose	<p>Spinosad, TGAI, 77.6% a.i.</p> <p>Dietary Concentrations: 0, 0.005, 0.015, 0.045, or 0.12% 0, 50, 150, 450, or 1200 ppm</p> <p>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</p> <p>Duration: 13 weeks (91 days) except for high dose, which was terminated on Day 44.</p>	<p>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.</p>	<p>Stebbins et al. 2002, Study 1</p> <p>Working Note: Except for minor differences in reporting and estimates of daily doses, this study appears to be identical to MRID 43566602.</p>
Rats, adult, male, Sprague-Dawley, 160-185 g, 10 per dose	<p>Spinosad (Tracer[®] formulation, 24% a.i., SC)</p> <p>In wheat grain Dietary Concentrations: 0, 8, and 16 ppm grain. Duration: 90 days</p> <p>Food consumption not reported.</p>	<p>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).</p>	<p>El-Hoda et al. 2012 Egypt</p>
Rats, Fischer 344, 10 per sex per dose.	<p>Spinosad, 77.6% a.i., 5:1::A:D.</p> <p>Dietary Concentrations: 0, 0.05, 0.1, 0.2, or 0.4% Dietary Concentrations: 0, 500, 1000, 2000, 4000 ppm.</p> <p>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</p> <p>Duration: 90 days Working Note: FAO review rounds doses to two significant places.</p>	<p>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).</p> <p>High Dose (4000 ppm): Discontinued on Day 44 due to deaths in 5/10 male and female rats.</p>	<p>MRID 43566601 U.S. EPA/OPP/HED 2009a</p> <p>Also summarized in FAO/WHO 2001.</p>

Appendix 1: Toxicity to mammals (*continued*)

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
<p>Rats, 10 per sex per dose. (neurotoxicity)</p>	<p>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: 0, 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.</p>	<p>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.</p>	<p>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.</p>
Chronic			
<p>Dogs, beagle, 4 per sex per dose</p>	<p>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</p>	<p>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.</p>	<p>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.</p>
<p>Dogs</p>	<p>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</p>	<p>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</p>	<p>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.</p>

Appendix 1: Toxicity to mammals (*continued*)

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
Mice, CD-1, 50 per sex per dose.	<p>Spinosad, TGAI, XDE-105, 88% a.i., 6.4:1::A:D.</p> <p>Dietary Concentrations: 0, 25, 80, or 360 ppm</p> <p>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.</p> <p>Duration: Up to 18 months. Interim sacrifices at 3 and 12 months.</p>	<p>NOAEL: 11.4 mg/kg/day in males, 13.8 mg/kg/day in females. Average of NOAELs: 12.6 mg/kg bw/day.</p> <p>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.</p> <p>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.</p>	<p>Bond et al. 1995a MRID 43701505 U.S. EPA/OPP/HED 1997b, 2009a</p> <p>Working Note: Classified as Supplemental in DER and HED 1997b but upgraded to Acceptable/Guideline in HED 2009a.</p> <p>Also published in Stebbins et al. 2002.</p>
Mice, CD-1, 60 per sex per dose	<p>Spinosad</p> <p>Dietary Concentrations: 0, 0.0008, or 0.024%</p> <p>Dietary Concentrations: 0, 8, 240 ppm</p> <p>Doses: (M/F): Male: 0, 1.1, or 32.7 mg/kg/day. Female: 0, 1.3, or 41.5 mg/kg/day.</p> <p>Duration: 18 months</p>	<p>NOAEL not established.</p> <p>LOAEL = 1.1 mg/kg/day in males; 1.3 mg/kg/day in females. No evidence of carcinogenicity.</p> <p>Working Note: This study is not discussed or explicitly discounted in EPA risk assessments although the study is classified as <i>Acceptable/Guideline</i>. FAO/WHO (2001) indicates that only <i>limited investigations were carried out on the low dose group</i>. No pathology is reported.</p>	<p>MRID 44123601 U.S. EPA/OPP/HED 2009a; 2010b</p> <p>Also summarized in FAO/WHO (2001) as Bond et al. 1996.</p>

Appendix 1: Toxicity to mammals (*continued*)

Organism	Agent/Exposure	Response	MRID, Study Date, Classification
<p>Rats, Fischer 334, 50 per sex per dose.</p> <p>Plus interim sacrifice group of 15 per sex per dose. See entry below.</p>	<p>Spinosad, XDE-105, 88% a.i., 6.4:1::A:D.</p> <p>Dietary Concentrations: 0, 0.005, 0.02, 0.05, or 0.1%</p> <p>Dietary Concentrations: 0, 50, 200, 500, or 1000 ppm</p> <p>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.</p> <p>Duration: 24 months</p>	<p><u>U.S. EPA/OPP/HED 2009a</u></p> <p>NOAEL = 9.5 mg/kg/day in males, 12.0 mg/kg/day in females. Average of NOAELs: 10.75 mg/kg bw/day.</p> <p>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.</p> <p><u>EPA Data Evaluation Record</u></p> <p>NOAEL: 3 mg/kg bw/day</p> <p>LOAEL: 9.5 mg/kg bw/day based on vacuolation of epithelial follicular cells of the thyroid in both sexes.</p> <p>High Dose: Significant increase in mortality and decrease in body weight in both males and females (Tables 3 and 4 of DER).</p> <p>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.</p> <p>This discrepancy does not impact the RfD (based on NOAEL of 2.49 mg/kg bw/day in dogs).</p> <p>Chronic phase of study classified as Supplementary in DER.</p>	<p>Bond et al. 1995b</p> <p>MRIDs 43701507 and 43710503</p> <p>U.S. EPA/OPP/HED 2009a</p> <p>Working Note: This study is not detailed in HED 1997b. The DER is dated March, 1996.</p> <p>Also published in Yano et al. 2002 and is summarized in detail in FAO/WHO 2001.</p>

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.

Appendix 1: Toxicity to mammals (*continued*)

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.	<p>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.</p> <p>NOAEL: 50 mg/kg bw/day (maternal and developmental).</p> <p>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 <i>toxicologically significant</i>.</p>	<p>Vedula et al. 1994 MRIDs 43414521 and 43770703 (range-finding) U.S. EPA/OPP/HED 1997b, 2009a</p> <p>Also published in Breslin et al. 2000.</p>
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.	<p>Maternal Effects (marginal): Decreased body weight gain and body weight for 1 day at the highest dose.</p> <p>NOAEL: 200 mg/kg bw/day (maternal and developmental).</p> <p>Given the higher sensitivity of rabbits (see above), the DER classifies this study as <i>Core</i>, even though no frank maternal toxicity was noted.</p>	<p>Liberacki et al. 1993 MRIDs 43557505 and 43770702 (range-finding) U.S. EPA/OPP/HED 1997b, 2009a</p> <p>Also published in Breslin et al. 2000. Abstract published in Marty et al. 1998</p>

Appendix 1: Toxicity to mammals (*continued*)

Species	Exposure	Response	MRID(s), (Year), Classification
Reproduction			
<p>Rat, Sprague Dawley, 6 weeks old (P₁) 30 per sex per dose</p>	<p>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: P₁: 10 weeks F_{1a} (P₂): 12 weeks. F_{1b} and F₂: not mated or otherwise exposed following birth.</p>	<p>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 P₁ and P₂ adults (see Figure 2 in Hanley et al. 2002). Thyroid hormone (thyroxin, T₄) levels: No effects in P₂ males or females at any doses (Table 4 of Hanley et al. 2002). U.S. EPA/OPP/HED (2009a, p. 5) indicates ... <i>increased levels of thyroid-stimulating hormone (TSH) and decreased levels of T₄.</i></p> <p>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 P₂ females.</p> <p>Offspring NOAEL: 10 mg/kg/day. LOAEL: 100 mg/kg/day based on decreases in litter size (F₂ only), survival and body weights.</p> <p>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.</p>	<p>Breslin et al. 1994 MRIDs 43701506 U.S. EPA/OPP/HED 1997b, 2009a</p> <p>Also published in Hanley et al. 2002</p>

Appendix 1: Toxicity to mammals (*continued*)

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.

Appendix 1: Toxicity to mammals (*continued*)

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.

Appendix 1: Toxicity to mammals (*continued*)

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

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

Species	Exposure	Response	Reference ^[1]
Mallard duck, <i>Anas platyrhynchos</i> , 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) <i>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 nominal 2000 mg/kg) (see p. 5 of DER)</i>	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, <i>Colinus virginianus</i> , 16-weeks-old, 222 ± 17 g (males), 214 ± 15 g (females), 6/dose group	Spinosad (88% a.i.) for 14 days <u>Nominal concentrations:</u> 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. <i>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 nominal 2000 mg/kg) (see p. 6 of DER)</i>	Murray et al. 1992b MRID 43414529 US EPA/OPP/EFED 2011a Supplemental Multiple dosing scheme employed in this study constitutes a major study deviation

Appendix 2: Toxicity to birds (*continued*)

Table A2-2: Acute Dietary Toxicity to Birds

Species	Exposure	Response	Reference ^[1]
Mallard duck, <i>Anas platyrhynchos</i> , 10-days-old, mean body weight: 148.2 ± 23.9 g, 10/dose group	<p>Spinosad (88% a.i.) for 5 days in the diet, followed by 3 days with untreated food</p> <p><u>Nominal concentrations:</u> 0, 75, 150, 300, 1250, 2500, or 5000 ppm a.i. (doses adjusted for purity of test substance)</p> <p><u>Measured concentrations:</u> 76.4, 151, 302, 1243, 2566, or 5156 ppm</p>	<p>No mortality or toxic effects</p> <p>LD₅₀ >5156 ppm [≈824 mg/kg bw^[1]]</p>	<p>Murray and Woolwine 1992 MRID 43414530</p> <p>US EPA/OPP/EFED 2011a</p> <p>Acceptable</p>
Northern bobwhite quail, <i>Colinus virginianus</i> , 13-days-old, mean body weight: 30.4 ± 2.7 g, 10/dose group	<p>Spinosad (88% a.i.) for 5 days in the diet, followed by 3 days with untreated food</p> <p><u>Nominal concentrations:</u> 200, 625, 1250, 2500, or 5000 ppm a.i. (doses adjusted for purity of test substance)</p> <p><u>Measured concentrations:</u> 210, 656, 1335, 2601, or 5253 ppm</p>	<p>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</p> <p>LD₅₀ >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]]</p> <p>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)</p>	<p>Murray et al. 1992a MRID 43414531</p> <p>US EPA/OPP/EFED 2011a</p> <p>Acceptable</p> <p>Note: Use approximate NOAEL of 200 mg/kg bw for acute risk characterization.</p>

^[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.

Appendix 2: Toxicity to birds (*continued*)

Table A2-3: Reproductive Toxicity in Birds

Species	Exposure	Response	Reference ^[1]
Reproduction			
Northern bobwhite quail, <i>Colinus virginianus</i> , 25-weeks-old, 1 male and 1 female/pen, 16 pens/group	Spinosad (88% a.i.) in diet for 21 weeks (1 generation) <u>Nominal concentrations:</u> 0, (acetone) 550, 1100, or 2200 ppm; corn oil vehicle <2% of diet	<u>At 550 ppm:</u> No apparent chronic or reproductive effects observed. <u>At 1100 ppm:</u> 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. <u>At 2200 ppm:</u> 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, <i>Anas platyrhynchos</i> , 26-weeks-old, 1 male and 1 female/pen, 16 pens/group	Spinosad (88% a.i.) in diet for 20 weeks (1-generation) <u>Nominal concentrations:</u> 0, (acetone) 550, 1100, or 2200 ppm; corn oil vehicle <2% of diet	<u>At 550 ppm:</u> No apparent chronic or reproductive effects observed. <u>At 1100 ppm:</u> 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. <u>At 2200 ppm:</u> 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.

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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, <i>Apis mellifera</i>	Spinosad (NOS) in acute oral toxicity test	LD ₅₀ = 0.057 µg/bee Duration not specified.	European Commission 2006
Honey bee, <i>Apis mellifera</i> (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, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , NOS	Spinosad (technical grade)	24-h LD ₅₀ : 0.06 µg/bee	Miles et al. 2002
Honey bee, <i>Apis mellifera</i> , 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

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Oral	Formulation		
Honey bee, <i>Apis mellifera</i>	Spinosad (NAF-85) in acute oral toxicity test	LD ₅₀ = 0.049 µg/bee	European Commission 2006
Honey bee, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , 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 = 0.0016-0.0031 µg 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: <i>Highly toxic to honey bees</i>	Hoxter et al. 1992 (DER) US EPA/OPP/EFED 2011a MRID 43414547 Acceptable Reported in Mayes et al. 2003 as 48 h-LD ₅₀ : 0.0024 µg/bee.
Honey bee, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , NOS	Spinosad (NOS) in acute contact toxicity test	LD ₅₀ = 0.0036 µg/bee	European Commission 2006
Honey bee, <i>Apis mellifera</i> , 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

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Honey bee, <i>Apis mellifera</i> , 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, <i>Apis mellifera</i> , 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 <i>Megachile rotundata</i> and <i>Nomia melanderi</i> in Table A3-2 below.	Mayer et al. 2001
Honey bee, <i>Apis mellifera</i>	Spinosad (technical 88% a.i.)	48 h-LD ₅₀ : 0.04 µg/bee	Mayes et al. 2003 citing unpublished report by Halsall and Grey 1998a. Also in Miles 2003 review.
Contact (µg/bee)	Formulations		
Honey bee, <i>Apis mellifera</i>	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 1998b.
Honey bee, <i>Apis mellifera</i>	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, <i>Apis mellifera</i>	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, <i>Apis mellifera</i>	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, <i>Apis mellifera</i>	1.6% WP formulation	24-h LD ₅₀ : 0.05 µg a.i./bee	Miles et al. 2002

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Contact (spray)			
Honey bee, <i>Apis mellifera</i> (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, <i>Apis mellifera</i> (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, <i>Apis mellifera</i> (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, <i>Apis mellifera</i> (NOS)	Spinosad (24% a.i.), no other exposure conditions specified. This is cited in the EFED 2011a document as a <i>field investigation</i> . 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

Appendix 3: Terrestrial Invertebrates (*continued*)

Table A3-2: Other Bees, Toxicity Values

Species	Exposure	Response	Reference
Acute Oral	Formulations		
Bumblebee, <i>Bombus terrestris</i> , 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, <i>Bombus terrestris</i> [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, <i>Melipona quadrifasciata</i> [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 <i>Melipona</i> 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

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Longer-term Oral	Formulations		
Stingless bee, <i>Melipona quadrifasciata</i> [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 to 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] LOAEL: 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, <i>Megachile rotundata</i> [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 <i>Apis mellifera</i> (Table A3-1) and <i>Nomia melanderi</i> (this table).	Mayer et al. 2001
Alkali bee, <i>Nomia melander</i> [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 <i>Apis mellifera</i> (Table A3-1) and <i>Megachile rotundata</i> (this table).	Mayer et al. 2001
Bumblebee, <i>Bombus terrestris</i> [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.

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Bumblebee, <i>Bombus terrestris</i> [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, <i>Bombus impatiens</i> [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, <i>Megachile rotundata</i> [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, <i>Osmia lignaria</i> [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, <i>Bombus terrestris</i> , 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, <i>Bombus terrestris</i> , 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.

Appendix 3: Terrestrial Invertebrates (*continued*)

Table A3-3: Other Arthropods, Toxicity Values

Species	Exposure	Response	Reference	
Acute Oral				
<i>Bactrocera dorsalis</i> , 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				
<i>Heliothis virescens</i> (larvae) [Lepidoptera : Noctuidae]	Spinosyn A	24-h LD ₅₀ : 0.014 (0.006–0.031) µg/larva	Salgado 1998	
<i>Periplaneta americana</i> , adults [Blattodea: Blattidae]	Spinosyn A	24-h LD ₅₀ : 0.74 (0.41–1.34) µg/animal	Salgado 1998	
<i>Periplaneta americana</i> , adults [Blattodea: Blattidae]	Spinosyn A	24-h LD ₅₀ : 1.9 µg/animal	Salgado et al. 1998	
Acute Contact				
<i>Aedes aegypti</i> , [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	
<i>Culex quinquefasciatus</i> [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	
<i>Anopheles quadrimaculatus</i> [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	
<i>Musca domestica</i> [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	
<i>Musca domestica</i> [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	
<i>Bactrocera dorsalis</i> , 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	
<i>Bactrocera dorsalis</i> , 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	
<i>Bactrocera cucurbitae</i> , melon fly [Diptera: Tephritidae]	Spinosad (Success SC 22.8% a.i.)	Duration	LD ₅₀	Hsu et al. 2012a
		(hours)	ng/fly	
		24	5.0	
		48	3.16	
		72	3.07	
Above are for laboratory strain. Resistance factors of up to about 5 (Table 2)				

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference												
<p><i>Manduca sexta</i>, 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.</p>	<p>Spinosad</p>	<table border="1" data-bbox="797 222 1149 384"> <thead> <tr> <th>Duration (hours)</th> <th>LD₅₀ ng/larva</th> <th>LD₅₀ mg/kg</th> </tr> </thead> <tbody> <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> </tbody> </table> <p>Use average body weight of 30 mg to estimate mg/kg bw dose. See Table 3 and Figure 3 of paper.</p>	Duration (hours)	LD ₅₀ ng/larva	LD ₅₀ mg/kg	24	59	1.97	48	2	0.067	72	0.4	0.013	<p>Herzog et al. 2002</p>
Duration (hours)	LD ₅₀ ng/larva	LD ₅₀ mg/kg													
24	59	1.97													
48	2	0.067													
72	0.4	0.013													
<p><i>Helicoverpa armigera</i>, cotton bollworm, late second or early third instar, 8–15 mg, [Lepidoptera: Noctuidae] 5 strains</p>	<p>Spinosad (NOS) Five concentrations, at least 24 larvae per dose.</p>	<p>48-h LD₅₀s: 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.</p> <p>Factor of 3.35 in variability appears to reflect simple variation in strains from different locations.</p> <p>A strain resistant to pyrethroids evidenced no cross-resistance to spinosad.</p>	<p>Achaleke et al. 2009</p>												
<p><i>Hyposoter didymator</i>, [Hymenoptera: Ichneumonidae] lepidopteran parasitoid</p>	<p>Tracer48 SC</p>	<p>48-h LD₅₀: 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. LD₅₀ based on 50% mortality in adults after emergence.</p>	<p>Schneider et al. 2003</p>												

Appendix 3: Terrestrial Invertebrates (*continued*)

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, <i>Apis mellifera</i>	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, <i>Apis mellifera</i> , 4 replicates, 30 bees per replicate Alfalfa leafcutter bee, <i>Megachile rotundata</i> , 4 replicates, 20 bees per replicate. Alkali bee, <i>Nomia melander</i> , 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

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
Honeybees, <i>Apis mellifera</i>	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)

Appendix 3: Terrestrial Invertebrates (*continued*)

Species	Exposure	Response	Reference
<p>Bumblebee (<i>Bombus impatiens</i>) colonies of 5-10 bees/colony. 7 colonies per dose group (including controls).</p>	<p>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</p> <p>Working Note: See discussion of application rates relative to residues on p. 3 of paper.</p> <p>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]</p> <p>Above are just crude approximations.</p>	<p>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</p>	<p>Morandin et al. 2005</p> <p>Primary literature. Simon Fraser University, Canada</p>
<p>Stingless bee (<i>Plebeia moureana</i>), 40 individuals trained for foraging experiment.</p>	<p>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.</p>	<p>No avoidance. No effect on foraging behavior.</p> <p>Working Note: Authors discuss that the short-term observations may have been inadequate to assess longer-term effects on foraging activity.</p>	<p>Sanchez et al. 2012</p> <p>Primary literature. Mexico. Public research center.</p>
Field Studies			
<p>Honeybees, <i>Apis mellifera</i>. 2 hives per treatment block (3.2 ha), 4 blocks.</p>	<p>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.</p>	<p>No effects on brood numbers (Table 2 of paper) or subjective assessments of colony health (Table 3 of paper).</p>	<p>Burns et al. 2001</p> <p>Primary literature. USDA in cooperation with Dow AgroSciences.</p>

Appendix 3: Terrestrial Invertebrates (*continued*)

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): <i>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.</i>	Mayes et al. 2003 citing unpublished study by Goodwin and Haine 1998.

Appendix 3: Terrestrial Invertebrates (*continued*)

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, <i>Apis mellifera</i>	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

Appendix 3: Terrestrial Invertebrates (*continued*)

Table A3-5: Toxicity to Earthworms

Species	Exposure	Response	Reference
Acute			
Earthworm, <i>Eisenia foetida</i>	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, <i>Eisenia foetida</i>	Spinosyn B	LC ₅₀ >1000 mg/kg soil (corrected value: >500 mg/kg soil)	European Commission 2006
Earthworm, <i>Eisenia foetida</i>	N-Demethylated spinosyn D	LC ₅₀ >1000 mg/kg soil (corrected value: >500 mg/kg soil)	European Commission 2006
Reproduction			
Earthworm, <i>Eisenia foetida</i>	Spinosad, NAF-85	NOEC >2700 g as/ha (corrected value: 1350 g as/ha)	European Commission 2006
Earthworm, <i>Eisenia foetida</i>	Spinosyn B	NOEC ≥3.582 mg/kg soil (corrected value: ≥1.791 mg/kg soil)	European Commission 2006
Earthworm, <i>Eisenia foetida</i>	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

Table A4-1: Vegetative Vigor 214
 Table A4-2: Seedling Emergence Vigor 215

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, <i>Zea mays</i> (Poaceae) Oat, <i>Avena sativa</i> , (Poaceae) Wheat, <i>Triticum aestivum</i> (Poaceae) Onion, <i>Allium cepa</i> (Liliaceae)	Spinosad, formulated product (44.2% a.i.), 0.5 lb a.i./acre	No phytotoxic or other effects <i>Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required</i>	U.S. EPA/OPP/EFED 2010a MRID 44597732 Acceptable
Dicots			
Carrot, <i>Daucus carota</i> (Apiaceae) Cucumber, <i>Cucumis sativus</i> (Cucurbitaceae) Radish, <i>Raphanus sativus</i> (Brassicaceae) Soybean, <i>Glycine max</i> (Fabaceae) Sunflower, <i>Helianthus annuus</i> (Asteraceae) Tomato, <i>Lycopersicon esculentum</i> (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 <i>Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required</i>	U.S. EPA/OPP/EFED 2010a MRID 44597732 Acceptable

Appendix 4: Toxicity to Terrestrial Plants (continued)

Table A4-2: Seedling Emergence Vigor

Species	Exposure	Response	Reference ^[1]
Monocots			
Corn, <i>Zea mays</i> (Poaceae) Oat, <i>Avena sativa</i> , (Poaceae) Wheat, <i>Triticum aestivum</i> (Poaceae) Onion, <i>Allium cepa</i> (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 <i>Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required</i>	U.S. EPA/OPP/EFED 2010a MRID 43701506 Acceptable
Dicots			
Carrot, <i>Daucus carota</i> (Apiaceae) Cucumber, <i>Cucumis sativus</i> (Cucurbitaceae) Radish, <i>Raphanus sativus</i> (Brassicaceae) Soybean, <i>Glycine max</i> (Fabaceae) Sunflower, <i>Helianthus annuus</i> (Asteraceae) Tomato, <i>Lycopersicon esculentum</i> (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 <i>Based on these results, terrestrial plant risk is considered minimal, and further plant tests (Tier II) are not required</i>	U.S. EPA/OPP/EFED 2010a MRID 43701506 Acceptable

Appendix 5: Toxicity to fish.

Table A5-1: Acute Toxicity in fish..... 216

Table A5-2: Longer-term toxicity in fish..... 219

Table A5-1: Acute Toxicity in fish

Species	Exposure	Response	Reference
Technical Grade			
Bluegill sunfish, <i>Lepomis macrochirus</i> , approximately 11-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 <u>Nominal concentrations:</u> 1.0, 2.5, 5.0, 6.5, 8.0, or 9.5 mg/L <u>Mean measured concentrations:</u> 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, <i>Cyprinus carpio</i> , juveniles, 10/treatment	Spinosad (NOS), under static conditions <u>Mean measured concentrations:</u> 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, <i>Cyprinus carpio</i>	Spinosad (NOS) for 96 hours	96-hour LC ₅₀ = 4.0 mg as/L (nominal)	European Commission 2006
Rainbow trout, <i>Oncorhynchus mykiss</i> , 10/treatment	Spinosad, technical grade (88% a.i.) under static conditions for 96 hours <u>Mean measured concentrations:</u> 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, <i>Cyprinodon variegatus</i> , 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 <u>Nominal concentrations:</u> 0, 1.6, 2.6, 4.3, 7.2, or 12 mg/L <u>Mean measured concentrations:</u> 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, <i>Cyprinus carpio</i>	NAF -85	96-hour LC ₅₀ >49 mg a.i./L (nominal)	European Commission 2006
Coho salmon, <i>O. kisutch</i> , 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. <u>Nominal concentrations</u> 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, <i>Oreochromis niloticus</i> , 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, <i>Oreochromis niloticus</i> , 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, <i>Oreochromis niloticus</i> , 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
<p>Nile tilapia, <i>Oreochromis niloticus</i>, juveniles, 11.55 ± 1.2g, 9.28 ± 0.54 cm, 6 fish/group, 3 replicates</p>	<p>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</p>	<p>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.</p>	<p>Pine and Uner 2014 Turkey</p>
<p>Zebrafish (<i>Danio rerio</i>), embryo-larvae, 2-4 replicates of 6 larvae/replicate</p>	<p>SpinTor™ 0, 0.2, 0.75, 2.0, 3.0, 7.5, or 30 ppb for 7 days. <u>Exposure:</u> 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.</p>	<p>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</p>	<p>Elskus 2007 USGS and Maine Department of Environmental Protection</p>

Appendix 5: Toxicity to fish (*continued*)

Table A5-2: Longer-term toxicity in fish

Species	Exposure	Response	Reference
<p>Mosquito Fish, <i>Poecilia reticulata</i>, adults, mean length 3.5 ± 0.2 cm, 21 fish/group</p> <p>Mature fish</p>	<p>Spinosad (NOS) 0, 60, 123, or 361 µg/L in static renewal test.</p> <p>Duration: 28 days</p> <p>Micronucleus assay</p>	<p>Genotoxicity manifested by inhibition of mitotic division, which the authors state could affect growth of the exposed fish.</p> <p>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).</p> <p>No indication of cytotoxicity.</p> <p>Working Note: The effects were not concentration related (See Figure 1 of paper). No marked temporal effect.</p>	<p>Anogwih et al. 2003</p> <p>Nigeria</p>
<p>Rainbow trout, <i>Oncorhynchus mykiss</i>, NOS</p> <p>Age of fish not specified.</p>	<p>Spinosad, NOS, under flow-through conditions for 21 days</p> <p><u>Mean measured concentrations:</u> 0, 0.63, 1.2, 2.1, 3.7, 6.0, or 10.2 mg/L</p>	<p>21-day LC₅₀ = 4.8 mg/L NOEC = 1.2 mg/L LOAEC = 2.1 mg/L</p>	<p>Cleveland et al. 2002b (DOW ERA)</p> <p>Not summarized in EPA risk assessments.</p>
<p>Rainbow trout, <i>Oncorhynchus mykiss</i>, embryos 2- to 24-hours old, 4 replicates, 50 embryos/replicate reduced to 25 embryos/replicate on Day 17.</p> <p>Early life-stage</p>	<p>Spinosad, technical grade (88% a.i.) in early life stage study under flow-through conditions.</p> <p><u>Nominal concentrations:</u> 0, 0.25, 0.50, 1.0, 2.0, 4.0, or 8.0 mg/L</p> <p><u>Mean measured concentrations:</u> 0.251, 0.498, 0.962, 1.89, 3.79, or 7.81 ppm.</p> <p>Duration:</p>	<p>No statistically significant differences observed for % embryos hatched, % normal larvae at hatch, or % survival to thinning.</p> <p>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.</p> <p>NOAEC = 0.498 ppm LOAEC (hatching) = 0.962 ppm LOAEC (survival) = 1.89 ppm LOAEC (body length) = 3.76 ppm</p>	<p>Weinberg et al. 1993 (DER)</p> <p>U.S.EPA/OPP/EFED 2011a MRID 43414541 Acceptable</p> <p>Also summarized Cleveland et al. 2002b.</p>

Appendix 5: Toxicity to fish (*continued*)

Species	Exposure	Response	Reference
<p>Sheepshead minnow, <i>Cyprinodon variegatus</i>, 40/replicate</p> <p>Early life-stage</p>	<p>Spinosad, technical grade (88% a.i.) for 37 days under flow-through conditions</p> <p><u>Mean measured concentrations</u>: 0.511, 1.15, 2.38, 4.84, or 9.63 mg/L</p>	<p>NOAEC = 1.15 ppm LOAEC (reduced growth) = 2.38 ppm</p>	<p>US EPA/OPP/EFED 2011a MRID 44420601 Acceptable</p> <p>Also summarized Cleveland et al. 2002b (DOW ERA)</p>
<p>Zebrafish, <i>Danio rerio</i>, embryo-larvae, 2-4 replicates of 6 larvae/replicate</p> <p>Early life-stage</p>	<p>SpinTor™ 0, 0.2, 0.75, 2.0, 3.0, 7.5, or 30 ppb for 7 days.</p> <p><u>Exposure</u>: 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.</p>	<p>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</p>	<p>Elskus 2007</p> <p>USGS and Maine Department of Environmental Protection</p>

Appendix 6: Toxicity to aquatic invertebrates

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Table A6-1: Acute Toxicity in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
Water flea, <i>Daphnia magna</i> , 24-hour-old instars	Spinosad with 50:50 mixture of spinosad A: Spinosad D for 48 hours <u>Mean measured concentrations:</u> 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, <i>Daphnia magna</i> , NOS	Spinosad (NOS) for 48 hours	48-hour EC ₅₀ >1.0 mg as/L (nominal)	European Commission 2006
Water flea, <i>Daphnia magna</i> , NOS	NAF-85 (NOS) for 48 hours	48-hour EC ₅₀ = 9.1 mg as/L	European Commission 2006
Water flea, <i>Daphnia magna</i> , <24 hours at initiation, 20/test concentration, 2 replicates/treatment level	Spinosad, technical grade (88% a.i.) under static conditions for 96 hours <u>Nominal concentrations:</u> 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 <u>Mean measured concentrations:</u> 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, <i>Daphnia magna</i> , 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. <u>Nominal concentrations:</u> 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, <i>Daphnia pulex</i> , 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. <u>Nominal concentrations</u> 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, <i>Ceriodaphnia dubia</i> , 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. <u>Nominal concentrations</u> : 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, <i>Daphnia magna</i>	Spinosyn A (99%)	EC ₅₀ : >197 mg/L Practically nontoxic	U.S. EPA/OPP/EFED 2009a MRID 46505307 Acceptable
Water flea, <i>Daphnia magna</i>	Spinosyn D (100%)	EC ₅₀ : 66.8 mg/L Slightly toxic	U.S. EPA/OPP/EFED 2009a MRID 46505309 Acceptable
Water flea, <i>Daphnia magna</i>	Spinosyn D (96%)	EC ₅₀ : 3.8 mg/L Moderately toxic	U.S. EPA/OPP/EFED 2009a MRID 46505304 Acceptable
Metabolites			
Water flea, <i>Daphnia magna</i>	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, <i>Daphnia magna</i>	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, <i>Daphnia magna</i>	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, <i>Daphnia magna</i>	B-13-14-Dihydropseudoaglycone 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, <i>Daphnia magna</i>	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, <i>Daphnia magna</i>	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, <i>Aedes aegypti</i> , larvae, late 3 rd instars	Commercial formulation, (Tracer® Naturalyte Insect Control), 480 g/L spinosad <u>Concentrations:</u> 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, <i>Aedes aegypti</i> , larvae, late 3 rd instars raised to adults post exposure	Commercial formulation, (Tracer® Naturalyte Insect Control), 480 g/L spinosad <u>Spinosad concentration:</u> 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

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response			Reference
Mosquitoes, <i>Culex pipiens</i> L., late 3 rd and early 4 th 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, <i>Aedes aegypti</i> , larvae, late 3 rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	Strain	LC₅₀ (mg/L)		Darriet et al. 2005
		SS	0.35		
		RR	0.32		
		See Table 1 of paper for confidence intervals and LC ₉₅ values.			
Mosquitoes, <i>Anopheles gambiae</i> , larvae, late 3 rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	Strain	LC₅₀ (mg/L)		Darriet et al. 2005
		SS	0.01		
		RR	0.011		
		See Table 1 of paper for confidence intervals and LC ₉₅ values.			
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, late 3 rd instars, 5 lots per concentration	Spinosad, TGAI for 24 hours	Strain	LC₅₀ (mg/L)		Darriet et al. 2005
		SS	0.093		
		RR	0.12		
		See Table 1 of paper for confidence intervals and LC ₉₅ values.			
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, susceptible 2 nd and 4th instars	Spinosad, technical powder (90.4% a.i.) designated as <i>old batch (lot QG28160W10)</i>	Instar	24-hour LC₅₀ (mg a.i./L)	48-hour LC₅₀ (mg a.i./L)	Jiang and Mulla 2009
		2nd	0.021	0.019	
		4th	0.033	0.026	
		See Table 1 of study for confidence intervals and LC ₉₀ values			
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, susceptible 2 nd and 4th instars	Spinosad, technical powder (90.4% a.i.) designated as <i>new batch (lot RL02160W02)</i>	Instar	24-hour LC₅₀ (mg a.i./L)	48-hour LC₅₀ (mg a.i./L)	Jiang and Mulla 2009
		2nd	0.024	0.019	
		4th	0.031	0.027	
		See Table 1 of study for confidence intervals and LC ₉₀ values			
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, susceptible 2 nd and 4th instars	Spinosad larvicidal liquid 120 SC (11.6% a.i.)	Instar	24-hour LC₅₀ (mg a.i./L)	48-hour LC₅₀ (mg a.i./L)	Jiang and Mulla 2009
		2nd	0.012	0.010	
		4th	0.014	0.013	
		See Table 1 of study for confidence intervals and LC ₉₀ values			

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference												
Mosquitoes, <i>Culex quinquefasciatus</i> , susceptible strain (Sebring-S) and field collected, larvae, 3 rd 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 <u>Sebring-S strain (reference):</u> LC ₅₀ = 0.028 ppm Resistance factor: ≈1.1	Jones and Ottea 2013												
Mosquitoes, <i>Aedes albopictus</i> , larvae, 4 th 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 pressure.	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 chlorpyrifos, cypermethrin, deltamethrin, lambda-cyhalothrin, and emamectin benzoate.	Khan et al. 2011a Pakistan												
Mosquitoes, <i>Aedes aegypti</i> , pupae and larvae (1 st to 4 th instars), 25/test	Spinosad, Obtained from T-Stanes & Company Limited, Research and Development Centre, Coimbatore, Tamil Nadu, India. Not clear if TGAI or formulation. <u>Concentrations:</u> 20, 40, 60, 80, or 100 ppm for 24 hours Study concerned with the larvicidal and pupicidal properties of spinosad against chikungunya vector	<table border="1"> <thead> <tr> <th>Life stage</th> <th>LC₅₀ (ppm)</th> </tr> </thead> <tbody> <tr> <td>1st Instar</td> <td>51.76882</td> </tr> <tr> <td>2nd Instar</td> <td>61.87610</td> </tr> <tr> <td>3rd Instar</td> <td>74.07166</td> </tr> <tr> <td>4th Instar</td> <td>82.18527</td> </tr> <tr> <td>Pupa</td> <td>93.44808</td> </tr> </tbody> </table> <p>See Table 2 for percent mortality, LC₉₀ values and confidence intervals. Working Note: The units of mg/L are correct.</p>	Life stage	LC ₅₀ (ppm)	1 st Instar	51.76882	2 nd Instar	61.87610	3 rd Instar	74.07166	4 th Instar	82.18527	Pupa	93.44808	Kovendan et al. 2012
Life stage	LC ₅₀ (ppm)														
1 st Instar	51.76882														
2 nd Instar	61.87610														
3 rd Instar	74.07166														
4 th Instar	82.18527														
Pupa	93.44808														
Mosquitoes, <i>Anopheles stephensi</i> , larvae/pupae laboratory colony	Spinosad, from Kalpatharu pesticide Limited, India. <u>Concentrations:</u> 0.01, 0.02, 0.04, 0.06, or 0.08 ppm 24 hour exposure	<table border="1"> <thead> <tr> <th>Life stage</th> <th>24 h-LC₅₀ (ppm)</th> </tr> </thead> <tbody> <tr> <td>1st Instar</td> <td>0.002</td> </tr> <tr> <td>2nd Instar</td> <td>0.003</td> </tr> <tr> <td>3rd Instar</td> <td>0.028</td> </tr> <tr> <td>4th Instar</td> <td>0.049</td> </tr> <tr> <td>Pupa</td> <td>0.030</td> </tr> </tbody> </table> <p>See Table 1 for percent mortality, LC₉₀ values and confidence intervals</p>	Life stage	24 h-LC ₅₀ (ppm)	1 st Instar	0.002	2 nd Instar	0.003	3 rd Instar	0.028	4 th Instar	0.049	Pupa	0.030	Kumar et al. 2011
Life stage	24 h-LC ₅₀ (ppm)														
1 st Instar	0.002														
2 nd Instar	0.003														
3 rd Instar	0.028														
4 th Instar	0.049														
Pupa	0.030														

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response			Reference	
Mosquitoes, <i>Culex quinquefasciatus</i> , HAmCq, MAmCq, and VBFmCq strains, larvae, 4 th instars, n=20	Spinisad (88% a.i.) for 24 hours. Wild caught strains versus laboratory (S-Lab) strain. No additional resistance pressure.	Strain	24-hour LC₅₀ (ppm)	Resistant Ratio	Liu et al. 2004a Alabama	
		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						
Mosquitoes, <i>Aedes albopictus</i> , HAmAal, MAmAal, VBFmAal, and SFmAal strains, larvae, 4 th instars, n=20	Spinisad (88% a.i.) for 24 hours Wild caught strains versus laboratory (Ikaken) strain. No additional resistance pressure.	Strain	24-hour LC₅₀ (ppm)	Resistant Ratio	Liu et al. 2004b Alabama	
		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						
Mosquitoes, <i>Culex pipiens</i> , larvae (4 th instar)	Spinisad (Tracer® 12% SC) for 24 hours Test solutions prepared on the basis of a.i. content	24-hour LC ₅₀ : 0.087 mg/L See Table 1 for LC ₂₅ values and fiducial limits			Mansour et al. 2012	
Mosquitoes, <i>Aedes aegypti</i> , 25 late 3 rd and early 4 th 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, <i>Aedes aegypti</i> , <i>Anopheles stephensi</i> , and <i>Culex pipien</i> , 3 rd 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 LC₅₀ (mg/L)	48-hour LC₅₀ (mg/L)	Romi et al. 2006	
		<i>An. stephensi</i>	0.039	0.024		
		<i>Ae. aegypti</i>	0.0096	0.0070		
		<i>Cx. pipiens</i>	0.0064	0.0032		
See Table 1 of study for LC ₉₀ and LC ₉₉ values						

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference												
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, late 3 rd instars, n=25, 45 generations	<p>Natular XRG (2.5% a.i.), sand granules for 24 hours</p> <p>Study entailed successive selections for 45 generations (i.e., Selected Colony). Susceptibility changes in response to selection were determined every other generation.</p> <p>Selection pressure: LC₇₀₋₉₀ over 45 generations. Test period: 24 hours</p>	<p>With Selection Pressure: F₁: LC₅₀: 0.671 mg/L F₄₅: LC₅₀: 693.5 mg/L Resistance factor: 1033</p> <p>Wild Population (no selection pressure): F₁: LC₅₀: 0.250 mg/L F₄₅: LC₅₀: 0.490 mg/L Range of LC₅₀s: 0.196 to 0.490 mg/L. (factor of 2.5)</p> <p>Reference Lab Culture (no selection pressure): F₁: LC₅₀: 0.272 mg/L F₄₅: LC₅₀: 0.311 mg/L Range of LC₅₀s: 0.234 to 0.424 mg/L. (factor of 1.8)</p>	Su and Chen 2014b												
Mosquitoes, <i>Culex quinquefasciatus</i> , larvae, 3 rd instars, 25/ concentration	<p>Natular T30 (8.33% a.i.)</p> <p><u>Concentrations:</u> 0.0045, 0.0030, 0.0060, or 0.0250 ppm for 24 hours</p>	24-hour LC ₅₀ = 0.0067 ppm	Su et al. 2014												
Other Standard Lethality Studies															
Non-biting midge, <i>Chironomus circumdatus</i> , larvae/pupae laboratory colony	<p>Spinosad, from Kalpatharu pesticide Limited, India. Formulation or a.i. not specified.</p> <p><u>Concentrations:</u> 0.01, 0.02, 0.04, 0.06, or 0.08 ppm</p>	<table border="1" data-bbox="818 1121 1149 1373"> <thead> <tr> <th data-bbox="818 1121 980 1213">Life stage</th> <th data-bbox="980 1121 1149 1213">24-hour LC₅₀ (ppm)</th> </tr> </thead> <tbody> <tr> <td data-bbox="818 1213 980 1245">1st Instar</td> <td data-bbox="980 1213 1149 1245">0.009</td> </tr> <tr> <td data-bbox="818 1245 980 1276">2nd Instar</td> <td data-bbox="980 1245 1149 1276">0.015</td> </tr> <tr> <td data-bbox="818 1276 980 1308">3rd Instar</td> <td data-bbox="980 1276 1149 1308">0.032</td> </tr> <tr> <td data-bbox="818 1308 980 1339">4th Instar</td> <td data-bbox="980 1308 1149 1339">0.053</td> </tr> <tr> <td data-bbox="818 1339 980 1373">Pupa</td> <td data-bbox="980 1339 1149 1373">0.049</td> </tr> </tbody> </table> <p>See Table 1 for percent mortality, LC₉₀ values and confidence intervals</p>	Life stage	24-hour LC ₅₀ (ppm)	1 st Instar	0.009	2 nd Instar	0.015	3 rd Instar	0.032	4 th Instar	0.053	Pupa	0.049	Kumar et al. 2011
Life stage	24-hour LC ₅₀ (ppm)														
1 st Instar	0.009														
2 nd Instar	0.015														
3 rd Instar	0.032														
4 th Instar	0.053														
Pupa	0.049														
Eastern oyster, <i>Crassostrea virginica</i> ,	<p>Spinosad, TGAI, for 96 hours under continuous flow conditions</p> <p><u>Mean measured concentrations:</u> 0, 0.093, 0.114, 0.222, 0.333, or 0.527 mg/L</p>	96-hour EC ₅₀ = 0.3 ppm (based on new shell growth)	<p>US EPA/OPP/EFED 2011a MRID 43444104/43571203 Acceptable</p> <p>Cleveland et al. 2002b</p>												
Amphipod, <i>Leptocheirus plumulosus</i>	Spinosad, TGAI, whole sediment toxicity study for 10 days	<p>Pore water NOAEC = 1.38 mg total residue/L</p> <p>Sediment NOAEC = 115 mg total residue/L</p>	U.S. EPA/OPP/EFED 2011a MRID 47702901 Supplemental												

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference
Grass shrimp, <i>Palaemonetes pugio</i> , NOS	Spinosaad, 87.9% a.i., 96 hours under static renewal conditions <u>Mean measured concentrations:</u> 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, <i>Simulium</i> spp., late-instar larvae 20/concentration	SpinTor 12SC <u>Nominal concentrations:</u> (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 (<i>Ischnura</i> sp., n=38); Dragonflies, (<i>Pachydiplax</i> <i>longipennis</i> , n=28); and Mayflies (<i>Caenis</i> sp., n=29) <i>Representative nontarget organisms based on abundance at collection site.</i>	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 damselflies., and then dragonflies See Figure 2 of study for mean mortality values indicated by bars representing concentrations.	Jones and Ottea 2013

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Table A6-2: Chronic toxicity in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
Spinosad			
Water flea, <i>Daphnia magna</i> , Instars, <24-hours-old, 5/replicate, 4 replicates	Spinosad (88% a.i.) under flow-through conditions for 21 days <u>Nominal concentrations:</u> 0, (solvent control), 0.6, 1.1, 1.7, 2.8, 4.6, or 7.7 µg/L <u>Mean measured concentrations:</u> 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 <u>LOAEC for specific effects:</u> 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, <i>Daphnia magna</i> , NOS	Spinosad, NOS, under flow-through conditions (5-day pulsed) <u>Mean measured concentrations:</u> 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, <i>Daphnia magna</i> , 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, <i>Daphnia magna</i> , <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, <i>Daphnia pulex</i> , <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, <i>Daphnia magna</i> , 4-6 th instars (<24-hours-old)	Spinosad, Conserve® 120 SC (11.6% a.i.) for 14 days <u>Nominal concentrations:</u> 2, 4, or 8 µg/L <u>Average exposure concentrations:</u> 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, <i>Daphnia pulex</i> , neonates, 30/concentration	<p>Spinosad (Success[®]), 240 g a.i./L for 8 days</p> <p>Concentrations: 0, 2, 4, 6, 8, 10, and 11 µg a.i./L. Static renewal (every other day).</p> <p>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).</p>	Concentration related decrease in populations (several metameters) at all concentrations (Figures 1 to 4 of paper).	Stark and Vargas 2003
Water flea, <i>Daphnia pulex</i> , 4-6 th brood offspring (<24-hours-old)	<p>Spinosad, Conserve[®] 120 SC (11.6% a.i.) for 14 days</p> <p><u>Nominal concentrations</u>: 2, 4, or 8 µg/L</p> <p><u>Average exposure concentrations</u>: 0.23, 0.50, or 0.62 µg/L</p>	Decreases in numbers of adults and offspring from Day 8 to Day 12 at all concentrations.	Duchet et al. 2011
Water fleas, <i>Daphnia pulex</i> , n=300	Spinosad (NOS) Exposure to 129 µg/L for 10 days	No organisms survived. No reproduction.	Stark 2005
Water flea, <i>Ceriodaphnia dubia</i> , ≤24-hours-old, 20/concentration	<p>Spinosad (Success[®]), 240 g a.i./L for 8 days</p> <p><u>Nominal concentrations</u>: 0, 0.5, 1, 2.5, or 10 µg a.i./L</p> <p>Authors indicate that spinosad appears to adversely affect <i>C. dubia</i> at or near the expected environmental concentration of 2.3µg/L.</p> <p>Working Note: Estimated upper bound concentrations in current risk assessment are higher than 2.3 µg/L</p>	<p>0.5 µg/L: NOAEC.</p> <p>≥1.0 µg/L Significantly reduced the final number of individuals, and population growth rate.</p> <p>≥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.</p> <p>10 µg/L: Population decline</p>	<p>Deardorff and Stark 2011</p> <p>Washington State University, Sponsored by NOAA</p> <p>This study is not summarized in ECOTOX.</p>

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference
Metabolites			
Water flea, <i>Daphnia magna</i> , 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, <i>Daphnia magna</i> , 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, <i>Daphnia magna</i> , 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, <i>Daphnia magna</i> , 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, <i>Chironomus riparius</i> , 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, <i>Chironomus riparius</i> , NOS	Spinosad, NOS, for 25 days under static conditions <u>Nominal concentrations:</u> 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, <i>Chironomus riparius</i> , 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, <i>Chironomus riparius</i> , 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 <i>Toxicity values based on mean-measured pore water treatment concentrations</i>	US EPA/OPP/EFED 2011a MRID 46505314 Non-guideline
Metabolites			
Freshwater midge, <i>Chironomus riparius</i> , larvae	Spinosyn B for 28 days	NOEC = 0.0032 mg/L (initial measured concentration in overlying water)	European Commission 2006
Freshwater midge, <i>Chironomus riparius</i> , 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. <i>Toxicity values based on mean-measured pore water treatment concentrations</i>	US EPA/OPP/EFED 2011a MRID 46505315 Non-guideline
Freshwater midge, <i>Chironomus riparius</i> , 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, <i>Chironomus riparius</i> , 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, <i>Chironomus riparius</i> , 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 <i>Toxicity values based on mean-measured pore water treatment concentrations</i>	US EPA/OPP/EFED 2011a MRID 46505316 Non-guideline
Freshwater midge, <i>Chironomus riparius</i> , NOS	β -13,14-Dihydropseudoaglycone of Spinosyn D for 28 days	NOEC \geq 0.731 mg as/L (mean measured initial concentration in overlying water)	European Commission 2006
Freshwater midge, <i>Chironomus riparius</i> , 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 μ g a.i./L for spinosad. LOAEC > 0.41 ppb a.i. EC ₅₀ > 0.41 ppb a.i. <i>Toxicity values based on mean-measured pore water treatment concentrations</i>	US EPA/OPP/EFED 2011a MRID 46505317 Non-guideline
Mosquitoes			
Mosquitoes, <i>Aedes aegypti</i> , 3 rd instar larvae, 25/concentration	Tracer EC (480 g a.i./L) concentration suspension for 10 days <u>Concentrations:</u> 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, <i>Aedes aegypti</i> , 4th instar larvae (24 hours after exposure of 3 rd instar larvae to spinosad) and 1-day-old pupae (96 hours after exposure of the 3 rd instar larvae), 20 larvae and 20 pupae/concentration	Tracer EC (480 g a.i./L) concentration suspension for 10 days <u>Concentrations:</u> 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 4 th instar larvae and pupae.	Tome et al. 2014
Mosquitoes, <i>Aedes aegypti</i> , five 4th instar larvae (24 hours after exposure of 3 rd instar larvae to spinosad) and five 1-day-old pupae (96 hours after exposure of the 3 rd instar larvae)	Tracer EC (480 g a.i./L) concentration suspension for 10 days <u>Concentrations:</u> 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 4 th instar larvae or pupae.	Tome et al. 2014
Shrimp			
Mysid shrimp, <i>Mysidopsis bahia</i> ,	Spinosad, TGAI for 28 days under flow-through conditions <u>Mean measured concentrations:</u> 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

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Table A6-3: Microcosm/Mesocosm Studies in Aquatic Invertebrates

Species	Exposure	Response	Reference
Daphnids			
<p>Water flea, <i>Daphnia pulex</i>, isolated natural populations.</p> <p>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.</p> <p>Microcosms contained algae (NOS), and mixed species of crustaceans (Table 1 of paper).</p>	<p>Spinosad 120 SC, 120 g a.i./L.</p> <p>Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment.</p> <p><u>Nominal concentrations for 30 cm water depth:</u> 8, 17, or 33 µg/L (5 replicates per concentration).</p> <p>Concentrations intended to reflect applications of 25, 50, or 100 g/ha. Concentrations based on nominal water depth of 30 cm</p>	<p>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).</p> <p>Working Note: Study authors note that the decreases in populations are not consistent with standard acute EC₅₀s. 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 <i>D. pulex</i> - i.e., LOAELs of 2 and 8 µg/L in Table 26 of current risk assessment.</p>	<p>Duchet et al. 2008</p>
<p>Water flea, <i>Daphnia magna</i>, isolated natural populations.</p> <p>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.</p>	<p>Spinosad 120 SC, 120 g a.i./L.</p> <p>Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment.</p> <p><u>Nominal concentrations for 30 cm water depth:</u> 8, 17, or 33 µg/L (5 replicates per concentration).</p> <p>Concentrations intended to reflect applications of 25, 50, or 100 g/ha. Concentrations based on nominal water depth of 30 cm.</p>	<p>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).</p> <p>Variations in water temperature and salinity had a significant effect on the abundance of <i>D. magna</i>. 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.</p> <p>Working Note: This study is consistent with LOAELs in <i>D. magna</i> from chronic studies. See Table 26 of current risk assessment.</p>	<p>Duchet et al. 2010a</p>

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference
<p>Other Invertebrates</p> <p>Non-biting midges, <i>Polypedilum nubifer</i> and <i>Tanytarsus curticornis</i> [Diptera: Chironomidae].</p> <p>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.</p>	<p>Spinosad 120 SC, 120 g a.i./L.</p> <p>Observations on Days 0, 2, 4, 7, 14 and 21 days after treatment.</p> <p><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.</p>	<p><i>Polypedilum nubifer:</i> Significant decrease in emergence starting on Day 4 at all concentrations (Table 1 of paper).</p> <p><i>Tanytarsus curticornis</i> 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.</p> <p>Working Note: The LOAEL of 8 µg/L is consistent with all of the chronic studies in <i>C. riparius</i>. See Table 26 of current risk assessment.</p>	<p>Duchet et al. 2015</p>
<p>Mosquitoes, <i>Culex tarsalis</i>, larvae (2nd instar) [Diptera: Culicidae] (target species) and chironomid midge larvae [Diptera: Chironomidae]</p> <p>1,150 liter mesocosms, mud substrate with added vegetation. 10-30 cm in depth</p>	<p><u>Natular® formulations of spinosad:</u> 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.</p> <p>Natular® G30 granules were applied by gloved hand at 14.57 kg/ha (13 lb/acre) – midrange of label rate</p> <p>Natular® 2EC liquid was applied by hand sprayer at 204.58 mL/ha (2.8 oz/acre) – maximum label rate</p>	<p>Both formulations of spinosad were highly effective against <i>Culex tarsalis</i>, larvae, and strongly suppressed chironomid midge larvae. Mortality rates of 52.4 to >96 % (Table 1 of paper)</p> <p>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.</p>	<p>Lawler and Dritz 2013</p>

Appendix 6: Toxicity to Aquatic Invertebrates (continued)

Species	Exposure	Response	Reference
<p>Mayflies, <i>Callibaetis californicus</i> [Ephemeroptera: Baetidae] nymphs, 3-5 cm long, 8-10/replicate</p> <p>1,150 liter mesocosms, mud substrate with added vegetation. 10-30 cm in depth</p>	<p>Natular® formulations of spinosad: G30 granules (sustained release) and 2EC liquid in 15 wetland mesocosms constructed in cattle watering tanks in Yolo County, CA.</p> <p>42 day observation period.</p> <p>Natular® G30 granules were applied by gloved hand at 14.57 kg/ha (13 lb/acre) – midrange of label rate</p> <p>Natular® 2EC liquid was applied by hand sprayer at 204.58 mL/ha (2.8 oz/acre) – maximum label rate</p>	<p>Mayfly nymphs were less sensitive than the target species, <i>Culex tarsalis</i> and midge larvae. Mortality rates of 26 to 94% (Table 1 of paper).</p> <p>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.</p> <p>Lesser sensitivity of Ephemeroptera relative to Diptera consistent with short-term study by Infante-Rodriguez et al. (2011).</p>	<p>Lawler and Dritz 2013</p>
<p>Mosquitoes, <i>Culex quinquefasciatus</i>, Diptera: Culicidae, natural populations of 3rd and 4th instars, but no pupae.</p> <p>Outdoor microcosms.</p>	<p>Spinosad 120 SC (11.6% a.i.) diluted with distilled water to 1.16% a.i.) in outdoor tubs.</p> <p>35 day observation period.</p> <p>Concentrations: 0.05, 0.1, 0.25, or 0.5 mg a.i./L</p> <p>Equivalent applications: 0.1, 0.2, 0.5, or 1 lb a.i./acre</p> <p>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.</p>	<p>Control of immature <i>Culex</i> 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.</p> <p>Working Note: This efficacy trial consistent with the acute LC₅₀ values from this study - i.e., 48 hour LC₅₀ values of about 0.01 mg a.i./L. for <i>C. quinquefasciatus</i>. See Table A6-1.</p>	<p>Jiang and Mulla 2009</p>
<p>Mosquitoes, <i>Culex quinquefasciatus</i>, Diptera: Culicidae, natural populations of early and late instars, and few pupae.</p> <p>Outdoor microcosms.</p>	<p>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.</p> <p>14 day observation period.</p> <p>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</p> <p>Sampling done by dipping technique before and 1, 4, 7, and 14 days after treatment to assess initial and persistent efficacy</p>	<p>Control of <i>Culex</i> mosquitoes for 14 days or longer at 0.025 to 0.1 mg a.i./liter.</p> <p>Working Note: This efficacy trial consistent with the acute LC₅₀ values from this study - i.e., 48 hour LC₅₀ values of about 0.01 mg a.i./L. for <i>C. quinquefasciatus</i>. See Table A6-1.</p>	<p>Jiang and Mulla 2009</p>

Appendix 7: Toxicity to Aquatic Plants

Table A7-1: Algae..... 238

Table A7-2: Macrophytes 239

Table A7-1: Algae

Species	Exposure	Response	Reference
Spinosad			
Freshwater diatom, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	Spinosad (NOS) for 120 hours (5 days)	EC ₅₀ = 0.079 mg a.i./L (mean measured)	European Commission 2006
Freshwater diatom, <i>Navicula pelliculosa</i>	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, <i>Skeletonema costatum</i>	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, <i>Anabaena flos-aquae</i>	Spinosad for 120 hours (5 days)	EC ₅₀ = 6.1 mg as/L (nominal)	European Commission 2006
Blue/green alga, <i>Anabaena flos-aquae</i>	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, <i>Selenastrum capricornutum</i>	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, <i>Selenastrum capricornutum</i>	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, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	β-13,14-dihydropseudo-aglycone of spinosad D for 96 hours	EC ₅₀ = 28 mg as/L (mean measured)	European Commission 2006
Freshwater diatom, <i>Navicula pelliculosa</i>	β-13,14-dihydropseudo-aglycone of spinosyn A for 72 hours	EC ₅₀ = 38.8 mg as/L (nominal)	European Commission 2006
Freshwater diatom, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	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, <i>Navicula pelliculosa</i>	N-demethylated spinosyn D for 120 hours	EC ₅₀ = 0.25 mg as/L (mean measured)	European Commission 2006
Freshwater diatom, <i>Navicula pelliculosa</i>	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, <i>Lemna gibba</i>	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

Appendix 8: Gleams-Driver Modeling, One Application

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)

Appendix 8: GLEAMS-Driver, Single Application

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)

Appendix 8: GLEAMS-Driver, Single Application

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)

Appendix 8: GLEAMS-Driver, Single Application

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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)

Appendix 9: GLEAMS-Driver, Two Applications (*continued*)

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.

Appendix 10: EPA Tier 1 Surface Water Models (continued)

FIRST Output Files

Single Application

CENTRAL ESTIMATE (Central Estimate of Koc)

```

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *
-----
RATE (#/AC) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM ) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
22.978 6.539
  
```

LOWER BOUND (Upper Bound of Koc)

```

RUN No. 1 FOR Spinosal ON None * INPUT VALUES *
-----
RATE (#/AC) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM ) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
16.963 0.523
  
```

UPPER BOUND (Lower Bound of Koc)

```

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *
-----
RATE (#/AC) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM ) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
43.404 11.001
  
```

Appendix 10: EPA Tier 1 Surface Water Models (continued)

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) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
45.955 13.078
* * * * *
  
```

LOWER BOUND (Upper Bound of Koc)

```

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *
-----
RATE (#/AC) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
33.926 1.045
* * * * *
  
```

UPPER BOUND (Lower Bound of Koc)

```

RUN No. 1 FOR Spinosad ON None * INPUT VALUES *
-----
RATE (#/AC) No.APPS & SOIL SOLUBIL APPL TYPE %CROPPED INCORP
ONE(MULT) INTERVAL Koc (PPM) (%DRIFT) AREA (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 DAYS UNTIL HYDROLYSIS PHOTOLYSIS METABOLIC COMBINED
(FIELD) RAIN/RUNOFF (RESERVOIR) (RES.-EFF) (RESER.) (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) ANNUAL AVERAGE (CHRONIC)
CONCENTRATION CONCENTRATION
-----
86.808 22.002
* * * * *
  
```

Appendix 10: EPA Tier 1 Surface Water Models (continued)

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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic 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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic 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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic metabolism (days)
1.000	1.0	1.000	8.31E+02	7300.0

groundwater screening cond (ppb) = 7.03E-01

* * * * *

Appendix 10: EPA Tier 1 Surface Water Models (continued)

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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic 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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic 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 rate (lb/acre)	Number of applications	Total Use (lb/acre/yr)	Koc (ml/g)	Soil Aerobic metabolism (days)
1.000	2.0	2.000	8.31E+02	7300.0

```
-----
```

groundwater screening cond (ppb) = 1.41E+00
