White Paper in Support of the Proposed Risk Assessment Process for Bees

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Executive Summary

Background

Over the past 60 years, the number of managed honey bee (*Apis mellifera*) colonies in the U.S. has been in decline and recent reports have indicated that the numbers of insect pollinators are in decline both in North America and Europe. A number of factors have been associated with declines in honey bees in the North America including disease, pests, poor nutrition, loss of habitat, pesticides and bee management practices; however, none of these factors have been identified as the cause. Researchers have hypothesized that some of these factors may interact and result in losses which are attributed to winter kill and Colony Collapse Disorder. Although pesticides have not been implicated as the singular cause of insect pollinator declines in general or of declines in honey bees specifically, efforts have been directed at determining the extent to which pesticides may be affecting bees and ways to mitigate potential effects. Regulatory authorities in North America and elsewhere are developing improved procedures for evaluating the potential risks of pesticides to bees.

While many pesticides are applied to foliar surfaces with the intention of affecting insects through contact, systemic pesticides can be applied to soil, seed or foliar surfaces and be transported to pollen and nectar where bees can be exposed through ingestion of residues. This paper considers exposures for foliar applied pesticides that may impact bees through contact and through the diet. This paper also addresses potential dietary exposures and effects of systemic pesticides on bees.

Although a number of insects provide pollination services, this white paper focuses on the honey bee. The honey bee is a social organism that lives in hives which can contain in upwards of 50,000 bees that are primarily worker bees (females) further subdivided by castes, but all under the direction of a single queen. The individual bee cannot function independent of the hive for prolonged periods; therefore, the functional unit of the honey bee is the colony itself. Given that the hive is composed of thousands of bees, it has been likened to a "superorganism". The attributes of the bee colony and its reliance on the queen represent challenges in assessing the potential risk of pesticides to bees since there can be complex routes of exposure and the colony can compensate for some losses given the redundancy that is built into the hive. Given the distance which bees can forage, spatial and temporal scales must be considered in evaluating potential exposure.

The extent to which regulatory authorities have evaluated the potential risks of pesticides to bees has varied; however, there are efforts both internationally and domestically to improve the process. In North America, regulatory agencies have had relatively similar processes. Toxicity tests used to evaluate potential effects have been tiered and have first examined effects on individual bees under laboratory conditions while more refined testing is conducted using whole hives under field conditions. These processes are continuing to evolve to reflect the changing science.

While EPA has evaluated the potential risks of pesticides to other taxa using a point estimate-based approach, it has historically only qualitatively described the potential hazard to bees. However, this paper describes a quantitative approach for developing risk quotients for bees. The proposed process is consistent with that used for other taxa in that it is both iterative and

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tiered and takes into account both foliarly acting pesticides as well as those which are distributed systemically in plants. Increased levels of refinement focus on areas where specific risks may exist and are intended to be increasingly representative of actual use/exposure conditions. As with other taxa, the risk assessment ultimately depends on a collective understanding of available data which includes information from registrant-submitted studies as well as information reported in the open literature and any data which may be available through incident reports.

There are several challenges that exist when integrating the various exposure and effects data that can be used to assess potential effects of pesticides on honey bees and their colonies. For instance, different bees are expected to be exposed to pesticides at different magnitudes, depending upon their function in the colony. In addition, interpreting the impacts of mortality and sublethal effects on the ultimate survival of the colony is complicated by a lack of definitive understanding of the linkages between many of these endpoints. Colony-level simulation models represent a useful tool that may be used to integrate exposure and effects data with the complexities of the social structure and biology of a honey bee colony.

Consistent with the existing process, the white paper is organized similar to the proposed risk assessment process and is divided into three sections, *i.e.*, problem formulation, analysis (exposure characterization and effects characterization) and risk characterization (estimation and discussion).

Problem Formulation

Problem formulation represents the initial phase of a risk assessment where management (protection) goals are defined and the subsequent assessment and measurement endpoints used in the assessment are articulated. This phase of a risk assessment also presents the conceptual models where the source of the stressor (pesticide use), routes of exposure, and effects (attribute changes) are also articulated. For bees, three protection goals are identified: pollination services, honey production and biodiversity. The primary assessment endpoints are colony survival, growth and reproduction and the proposed measurement endpoints include acute lethality to individual bees and measures of impaired survival, growth and reproduction of the colony itself. Several conceptual models are depicted to account for potential adverse effects from foliarly applied systemic and non-systemic pesticides and from systemic pesticides applied to soil, seeds or tree trunks.

Measures of effect are based primarily on honey bees because they are readily available, are relatively easy to work with under laboratory conditions and their husbandry needs are well documented both at the level of the individual bee as well as the colony. While the honey bee is the major focus of the process discussed in this paper, the honey bee is considered as a surrogate for other non-*Apis* bees even though many of these other insect pollinators are not social and have life histories which are substantially different from that of the honey bee.

Consistent with the process used for other terrestrial taxa (*e.g.*, birds and mammals), screening-level estimated environmental concentrations used for evaluating exposure to honey bees are based on an existing tools (*i.e.*, T-REX model) as well as approaches for estimating exposure through ingestion of pollen and nectar containing residues of a systemic pesticide applied to soil or seeds that have not previously been used for regulatory purposes in North America.

Characterization of Exposure

The proposed process for estimating exposure is consistent with that used for other taxa in that at a screening level (Tier I), exposure estimates are intended to be relatively conservative [high-end] while at higher tiers (Tiers II and III), exposure is based on measured values which are reflective of actual use conditions. While a number of routes of exposure are identified in the conceptual models depicted in the problem formulation, for pesticides that are applied via foliar spray, the primary routes of exposure are considered to be through contact and diet (*i.e.*, consumption of contaminated pollen or nectar) and for systemic pesticides applied to soil and seeds, the primary route of exposure is through the diet. With these methods, dose-based estimates of exposure through the diet are calculated by considering food consumption rates of larvae and adult worker bees and the estimated upper-bound concentrations in pollen and nectar. The larval consumption rate is based on daily food consumption rates of worker larvae during the last two days of the uncapped period, while the adult worker bee food consumption rate is based on that of nectar foraging worker bees. Due to their relatively high food intake rates of worker larvae and nectar foraging bees, these two daily food consumption rates represent the portions of the larval and adult life stages that are expected to receive the greatest pesticide exposures when considering the entire life cycle of a worker bee.

Different methods are presented to estimate pesticide concentrations in pollen and nectar for pesticide applications made via foliar spray, soil treatment, seed treatment and tree trunk applications. In screening-level assessments contact exposure is estimated for pesticides applied via foliar spray. An upper-bound residue value of chemicals on honey bees based on Koch and Weisser 1997 is proposed to represent contact exposures. The estimation of dietary exposure to pesticides applied via foliar spray, soil treatment, or seed treatment, involves a few different methods. The proposed methods described in this white paper differ in the nature of the estimated concentrations in pollen and nectar consumed by bees. For foliar spray applications, the proposed approach involves the use of the tall grass residue value from the T-REX model (v. 1.5) as a surrogate for pesticide concentrations in nectar and pollen. For soil treatments, the proposed Tier I method for estimating exposure involves the use of the Briggs' soil-plant uptake model, which is designed to estimate pesticide concentrations in plant shoots; these estimated concentrations in plant shoots are used as a surrogate for concentrations in pollen and nectar. For seed treatments, the proposed Tier I exposure method is based on the International Commission for Plant-Bee Relationships' (ICP-BR) 1 mg a.i./kg concentration as an upper-bound for pesticides in nectar and pollen.

Characterization of Effects

Since regulatory authorities have required pollinator toxicity testing for many years, multiple standardized tests exist that examine effects to both individual bees and colonies. The laboratory-based studies have historically focused on measuring effects in young adult bees; however, only recently have tests been developed to examine the effects of chemicals on larval bees. The proposed screening-level risk assessment process includes acute toxicity tests with adult and larval bees. Higher-tier assessments transition away from studies of individual bees to colony-level studies conducted under semi-field (tent/tunnel) and full field conditions. Toxicity tests conducted with both individual bees and colonies will continue to collect data on effects

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on survival for acute toxicity studies and on impaired survival, growth and reproduction for longer-term studies with whole colonies. Although additional endpoints (sublethal effects) may be measured in these studies, there can be considerable uncertainty in how these measurement endpoints relate to assessment endpoints and the whole organism adverse (apical) effects on which regulatory agencies are required to regulate. Until there are sufficient data to establish plausible adverse outcome pathways with consistent and reproducible linkages between molecular initiating events and key events across multiple levels of biological organization to an adverse effect at the whole organism/colony/population level, it is difficult to make use of sublethal effects other than in qualitatively describing potential adverse effects. In transitioning to higher tier testing, the resources involved in conducting and reviewing the studies also increase because the complexity of the study increases. Therefore, there is a need to refine the scope of these studies to address specific uncertainties which have been identified in lower-tier studies. As with refinements in exposure estimates, refined effects testing is intended to reflect increasingly realistic conditions surrounding how the chemical will actually be used. While relatively standardized test protocols exist for semi-field testing, full field studies are less well defined and would be requested on a case-by-case basis depending on the nature of uncertainties. Study design considerations are discussed for the conduct of these studies.

Risk Characterization

Risk characterization is intended to integrate estimates of exposure and effects to provide a quantitative estimate of potential risk. Consistent with the process used for other taxa, the proposed process determining risk to bees is based on the risk quotient (RQ) which is the ratio of point estimates of exposure to point estimates of effects. At a screening level the acute oral or contact lethal dose to 50% of the organisms tested (LD_{50}) serves as the denominator of the ratio while the contact or dietary exposure value serves as the numerator. The level of concern to which the acute RQ value is compared is set to 0.4 and is based on the historic average dose-response relationship for acute toxicity studies with bees and a 10% mortality level. When acceptable chronic toxicity test designs are available, the proposed process involves developing chronic RQs using NOAECs and evaluating the RQs using an LOC of 1. As with other taxa, the risk assessment process is intended to be iterative. Either through the incorporation of possible changes in use or through more refined estimates of exposure based on measured residue values, the RQ values can be further refined in the screening level to determine whether they exceed levels of concern. Further testing under semi- or full field conditions can provide additional information for qualitative evaluations of effects on the entire colony. These refinements would be considered in conjunction with any incident data available for the compound. Therefore, similar to the process used for other taxa, multiple lines of evidence are considered and integrated in the proposed risk assessment process for bees.

Although this white paper focuses on honey bees, the process described herein applies to both individual bees and to the colony and thereby is applicable to other insect pollinators, in particular non-*Apis* bees. The paper acknowledges the limitations in this assumption. While some toxicity testing methods may be available for evaluating effects to non-*Apis* bees, these tests have not been sufficiently vetted at this time to support their use in quantifying risks to these other taxa.

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Prologue

This white paper is intended to provide an introduction to the proposed risk assessment process for insect pollinators using honey bees (*Apis mellifera*) as a surrogate. The paper is divided into sections that are intended to be consistent with the risk assessment process described by the EPA Ecological Risk Assessment Guidelines (USEPA 1998) which is both tiered and iterative. After an introduction to issues associated with bees and a brief discussion about the biology of plants and bees relative to potential routes of exposure, the paper discusses a proposed risk assessment process consisting of screening level (Tier I) exposure and effects assessments including a description of how risks may be quantified using these data. While EPA has routinely required effect studies for bees to support its qualitative assessment of potential hazards to bees, a well-defined tiered process for evaluating exposure does not currently exist; therefore, considerably greater detail is devoted to the Tier 1 exposure section. The paper also discusses more refined assessments which transition away from data based on individual organisms, toward colony-based assessments under controlled test conditions where test colonies are confined to enclosures (Tier II semi-field studies) and then under less controlled, but more realistic conditions where bees are allowed to forage freely (Tier III full field studies). In addition, this paper discusses the potential utility of a colony-level model in integrating exposure and effects data collected to assess risks at some or all of the three tiers in order to assess the risks of a pesticide in impacting colony survival, pollination services and honey production. With increasing levels of refinement, both exposure and effect estimates are intended to be increasingly representative of how the pesticide is used. These refinements imply that exposure and effects study methods are directed at addressing specific uncertainties with an increasing level of realism. The proposed process is intended to enhance the ability of EPA's Office of Pesticide Programs, the Canadian Pest Management Regulatory Agency (PMRA) and the California Department of Pesticide Regulation (CalDPR) to reliably assess lists of chemicals for direct and indirect effects specifically on managed honey bee colonies as well as other non-*Apis* bees using honey bees as a surrogate and/or when appropriate data are available.

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List of Commonly Used Abbreviations and Nomenclature

μg/kg	Symbol for "micrograms per kilogram"
$\mu g \cdot L^{-1}$	Symbol for "micrograms per liter"
ϑ	soil-water content by volume (cm^3/cm^3)
δ	soil bulk density (g-dw/cm^3)
ρ_b	bulk density (kg/m^3)
θ_{sed}	Sediment porosity (unitless)
a.i.	Active Ingredient
AOP	Adverse Outcome Pathway
BCF	Bioconcentration Factor
BEAD	Biological and Economic Analysis Division
BFD	Brood Fixing Day
Bw	Body Weight
CCD	Colony Collapse Disorder
CFR	Code of Federal Regulations
CalDPR	California Department of Pesticide Regulation
CI	Confidence Interval
CL	Confidence Limit
$C_{dew(t)}$	Concentration in dew (mg a.i./L)
C_{soil}	concentration in soil (μg a.i./g soil)
C_{stem}	concentration in stems (μg a.i./g plant)
C_w	Concentration in water (μg a.i./L)
DACO	Data Code (Canadian Use Site Category)
d_{sed}	Depth of sediment (m)
d_w	Depth of water (m)
EC_{25}	25% Effect Concentration
EC_{50}	50% (or Median) Effect Concentration
ECOTOX	EPA managed database of ECOTOXicology data
EEC	Estimated Environmental Concentration
EFED	Environmental Fate and Effects Division
EFSA	European Food Safety Authority
e.g.	Latin *exempli gratia* ("for example")
EIIS	Ecological Incident Information System
EPPO	European and Mediterranean Organization for Plant Protection
et al.	Latin *et alii* ("and others")
Etc.	Latin *et cetera* ("and the rest" or "and so forth")

EU	European Union
FAO	Food and Agricultural Organization (United Nations)
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
f_{oc}	fraction of organic carbon in soil
g a.i./ha	grams of active ingredient per hectare
GENEEC	GENeric Estimated Environmental Concentration
Ha	hectare
HQ	Hazard Quotient
IAPV	Israeli Acute Paralysis Virus
ICP-BR	International Commission for Plant-Bee Relationships
i.e.	Latin for *id est* ("that is")
IEC	Individual Effect Chance Model (Version 1.1)
K_{CW}	Partitioning coefficient between plant cuticle and water
K_d	Solid-water Distribution Coefficient
K_f	Freundlich Solid-Water Distribution Coefficient
Kg	Kilogram(s)
km	Kilometer(s)
K_{oc}	Organic-carbon Partition Coefficient
K_{ow}	Octanol–water Partition Coefficient
L	Liter
L/kg-oc	Liters per kilogram organic carbon
lb a.i./A	Pound(s) of active ingredient per acre
LC_{50}	50% (or Median) Lethal Concentration
LD_{50}	50% (or Median) Lethal Dose
LOAEC	Lowest Observable Adverse Effect Concentration
LOC	Level of Concern
Log	Logarithm
LOQ	Level of Quantitation
m	meter(s)
m_{ai}'	mass applied per unit area (kg/ha)
mg	Milligram(s)
mg/kg	Milligrams per kilogram (equivalent to ppm)
mg/L	Milligrams per liter (equivalent to ppm)
mi	mile(s)
mL	milliliter
MOR	Magnitude of Residue studies
MRID	Master Record Identification Number

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n/a	Not applicable
NASS	National Agricultural Statistics Service
NOAEC	No Observable Adverse Effect Concentration
NPIC	National Pesticide Information Center
OCSPP	Office of Chemical Safety and Pollution Prevention (formerly OPPTS)
OECD	Organization for Economic Cooperation and Development
OEPP	Organisation Européenne et Méditerranéenne pour la Protection des Plantes (EPPO)
OPP	Office of Pesticide Programs
OPPTS	Office of Pollution Prevention and Toxic Substances
ORD	Office of Research and Development
PCA	Percent Cropped Area
PEIP	Pesticide Effects on Insect Pollinators (OECD)
pK_a	Symbol for the negative logarithm of the acid dissociation constant, dimensionless
PMRA	Pest Management Regulatory Agency (Canada)
ppb	Parts per Billion (equivalent to µg/L or µg/kg)
ppm	Parts per Million (equivalent to mg/L or mg/kg)
PPR	Plant Protection Products and the Residues (EPPO)
PROC NLIN	Non-linear regression procedure (SAS®)
PRZM	Pesticide Root Zone Model
RAC	Raw Agricultural Commodities
RQ	Risk Quotient
SAP	FIFRA Scientific Advisory Panel
SAS®	Statistical Analysis System
SCFs	Stem Concentration Factor(s)
SETAC	Society of Environmental Toxicology and Chemistry
T-REX	Terrestrial Residue Exposure model
TFD	Terrestrial Field Dissipation
TSCFs	Transpiration Stream Concentration Factor(s)
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

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1 Introduction

1.1 Overview of pollinator declines and factors associated with declines

A number of sources have reported declines in certain pollinator species globally. A 2006 report by the National Academies of Science (NAS 2008) indicated declines in some North American pollinators, including North America's most important managed pollinator, the honey bee (*Apis mellifera*). The report noted that insufficient information existed to determine the causes of those declines. Similar declines have also been reported in Europe in a report by J. C. Biesmeijer *et al.* 2006. The decline of managed honey bees in the United States has also been measured by the U.S. Department of Agriculture (USDA) National Agricultural Statistics Survey (NASS 2008). NASS data indicate that managed honey bee colonies have declined from a peak of approximately 6 million colonies in 1947 to roughly 2.5 million in 2006 (USDA 2012). According to USDA, since the introduction of parasitic mites into the U.S. in the 1980s, typical annual overwintering losses of managed honey bee colonies have been roughly 15% to 21% for beekeepers; however, annual overwintering colony losses ranging between 31% and 36% were reported in the United States between 2007 and 2010. In 2006, some commercial beekeepers reported losses of 30 – 90% of their hives during the wintering months. A portion (approximately 50%) of these declines were characterized by the disappearance of adult worker bees with few or no dead bees in the colony, the presence of capped brood with a small cluster of nurse bees, the queen, and the presence of intact honey and pollen stores; this syndrome has been termed Colony Collapse Disorder (CCD) (USDA 2007). Trends in overwintering losses in Canada are similar to those reported in the United States. Prior to the introduction of parasitic mites in Canada, normal long-term overwintering mortality has been considered to be 15%, while annual overwintering colony losses ranging between 21.0% and 35.0% were reported in Canada between 2006 and 2011 (CAPA 2010, 2011). Symptoms by which CCD is being characterized in the U.S. have not been diagnosed by professional apiculturists in Canada (CAPA 2010, 2011). Although a number of factors/agents have been hypothesized as potential contributors to CCD, increased overwintering losses, and to declines in honey bee health in general, at this time, no factor has been identified as the single cause. Rather, the available science suggests that pollinator declines are a result of multiple factors which may be acting in various combinations (Pettis and Delaplane 2010). Research is being directed at identifying the individual and combinations of stressors that are most strongly associated with pollinator declines.

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While the exact cause(s) of the general decline in pollinator species and the phenomenon characterized as CCD have not been determined, potential contributing factors including diseases, habitat destruction/urbanization, agricultural practices/monocultures, pesticides, nutrition, and bee management practices (USDA 2007) must be considered. Researchers at the USDA have hypothesized that CCD may be caused by primary stressors (*e.g.*, parasitic varroa mites (*Varroa destructor*), poor bee management, nutrition and/or pesticides that may in turn cause honey bees to become more susceptible to disease (Yang and Cox-Foster 2005, USDA 2007, vanEngelsdorp *et al.* 2008, vanEngelsdorp *et al.* 2009). Surveys of managed migratory bee colonies indicate that a broad range of pesticides have been detected in hive products (*e.g.*, honey, stored pollen, wax). The most frequently detected pesticides and the two that occur in the highest quantity are those used by beekeepers to control varroa mite (coumaphos and fluvalinate). Typically, a combination of pesticides is detected in the same hive products, with an average of four pesticides detected in the same sample (Mullin *et al.* 2011). In spite of the presence of these compounds in honey bee colonies, at this time, there has been no correlation between the incidences of CCD or pollinator declines in general, with use of any pesticide or class of pesticides (vanEngelsdorp *et al.* 2009).

Historically, pesticides and in particular insecticides, have been applied via foliar spray in order to kill target pests on contact. Models are currently available and are already used for regulatory purposes for estimating exposures of non-target animals to pesticides that have been applied via foliar spray (*i.e.*, T-REX). At this time, models are not being used in North America to estimate exposures to non-target animals when the pesticides are systemic and are applied to the soil, seeds or tree trunks. In considering the potential for pesticides to affect bees, increased attention has been devoted to dietary exposure of systemic pesticides to the honey bee. The following section provides a brief overview of how exposure to bees may occur via movement of systemic pesticides through plants.

1.2 Plant Sugar, Nutrient and Water Transport and the Movement of Systemic Compounds

Plants transport sugars, nutrients and water through specialized vascular tissues called xylem and phloem. Depending on physical-chemical properties, systemic pesticides can move within these vascular tissues to untreated tissues of the plant or remain locally distributed through extracellular movement.

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Therefore, a pesticide may be xylem mobile, phloem mobile, both xylem and phloem mobile, or locally systemic.

Xylem transports water and mineral nutrients from below-ground sources through the roots and upwards through the plant to the stems, leaves, flowers, and fruits (Raven *et al*. 1992). In order to enter the xylem located in the center of the root, water (containing minerals and pesticides) must pass through the endodermis[1]. Because the cell walls and the intercellular space between the endodermis cells are filled with suberin and lignin, they are impenetrable to nearly all compounds, thus requiring materials to pass through the living phospholipid membrane of the endodermis cells. Minerals and other compounds are selectively transported across the membrane by active transport. Once through the endodermis, the compound can move either within the living cells (protoplastic movement), or between them (apoplastic movement). The primary mechanism for moving water upward is passive and based on a negative water potential gradient created by the evaporation of water from stomata[2] that occurs because of evapotranspiration. As molecules of water leave the stomata, they pull on the entire water column within the xylem tissues, thus evaporation draws the water upwards. Xylem tissues are composed of non-living cells and are found throughout the plant, maintaining water pressure and nutrient flow to all living tissues, including flowers, anthers (pollen containing structures), fruits, and nectaries (Escalante-Pérez and Heil 2012). Chemicals that are transported through the xylem (acropetalous systemics) are then transported upwards with the flow of water such that if these chemicals are applied to the soil, the entire plant will be exposed. Foliar applications of xylem-transported chemicals that are not also transported within the phloem will result in the chemical remaining in the leaves and will not likely travel to the roots or later developing structures such as flowers or fruits unless the chemical also comes in contact with the stem.

Phloem is the living tissue that actively transports photosynthate (*i.e.*, sugars) to portions of the plant that are growing, storing starches, or exuding nectars. Sugar and other compounds transported through the phloem are mediated by bulk flow from the source in photosynthetic cells to the various sinks. Phloem transport of chemicals results in the movement of the photosynthate throughout all living tissues of a vascular plant regardless of the point of contact. Phloem transport generally occurs in a downward movement from the leaves toward non-photosynthetic tissues such as roots and storage

[1] a selective barrier of living cells with an impermeable intercellular space
[2] tightly regulated openings in the epidermis

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organs, but also includes movement to nectaries, and developing above-ground structures such as flowers, fruits and seeds. To enter the phloem through a soil application, a pesticide would require transport through the cortex cells and the regulatory endodermis. Once in the central vasculature of the root, the pesticide would be transported upwards with the water in the xylem; however it would also accumulate in phloem. Compounds that are likely to be captured or "trapped" by the phloem can become more concentrated in phloem than in xylem; these compounds would be of particular concern for nectar exposures. Unlike for xylem-only transported compounds, the foliar application of a phloem transported compound would result in chemical transport out of the leaf and into the roots, developing tissues, flowers, nectaries and fruits.

If a compound is unable to pass into the plant vascular system, the chemical could still be locally systemic. Locally systemic compounds can move to untreated tissues of the plant via apoplastic movement through diffusion between cells (*e.g.*, translaminar movement from the upper leaf surface to the underside of the leaf surface). This process would provide relatively low probability of transport out of the tissues where the chemical made contact.

Depending on their route of transport within the plant, systemic pesticides can move to untreated tissues of the plant via apoplastic movement, xylem transport, and/or phloem transport. With these three types of transport mechanisms, systemic pesticides may move within the extracellular matrix, move unidirectionally upwards through the xylem from the base of the plant to the exposed shoots (xylem transport), move unidirectionally downwards from the above ground tissues to the roots of the plant (phloem transport), or move bidirectionally. Bidirectionally transported chemicals, two-way systemic pesticides (acro/basipetalous transport), move through the xylem and phloem of the plants resulting in nearly complete integration throughout the plant tissues. The chemical/physical properties of a pesticide determine whether it is systemic, and the nature of its systemic movement, *i.e.* whether it will move from the tissues that were exposed into the roots, stems, leaves, pollen, nectar, fruit and/or seeds of the plant. Furthermore, pesticide residues in these tissues can be influenced by the growth stage of the plant, whereby tissue with a high nutrient need or greatest growth potential (*e.g.*, buds, flowers, tubers) will accumulate more sugars and could, by extension, accumulate higher residues of phloem mobile pesticides.

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1.2.1 Plant Structures that are Attractive to Bees

Honey bees primarily collect nectar and pollen from plants. In addition, they collect honey dew, plant waxes, resins and may collect guttation fluid. Because honey bees rely on nectar and pollen to meet the majority of their nutritional requirements, the focus of the proposed risk assessment method is on these two plant-based materials. Brief descriptions of the plant structures that house nectar and pollen are provided below.

Nectaries are diverse in their structural form (Escalante-Pérez and Heil 2012) but can be generally described as living tissues that release sugars, amino acids and other compounds to the surface for pollinator or defender attraction. Nectaries are found in flowers, as well as on leaves and stems (extrafloral nectaries), and release variably viscous mixtures of water, sugars, amino acids, phenolics and many other compounds. Nectar may be contaminated with pesticides applied via foliar spray when flowers are open and because extrafloral nectaries are located on the surface of the plant. When nectaries are producing exudates, the sugars come directly from phloem, resulting in a high likelihood of bee exposures to those pesticides that are phloem-transported. Potentially high exposure could result from compounds prone to phloem trapping because of the potential for greater concentrations to be found in phloem and nectar relative to pesticides within the xylem.

Pollen is contained in the anthers of flowers. Pollen can be contaminated by pesticides applied directly to open flowers (*i.e.,* through foliar spray applications). The development and maturity of pollen grains in the stamen (anthers) of flowering plants depends on nutrients and water transport and could lead to systemic pesticide exposure to bees. The stamen is comprised of two main structures, the filament and the anther, both containing xylem and phloem vascular tissues. During the development and maturation of pollen grains, they are surrounded by a solution of water, proteins, amino acids and many other chemicals, potentially including systemic pesticides transported by either xylem or phloem. During the maturation phase of development, the fluid matrix dries out and the materials in the solution are deposited onto the exterior surface of the pollen grain.

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1.3 Consideration of Honey bee Biology in Ecological Risk Assessment

A careful consideration of honey bee biology and ecology is necessary for developing ecological risk assessment methods that facilitate attainment of pollinator protection goals through appropriate risk management activities. In particular, honey bees possess a number of distinctive attributes which identify routes of exposure to pesticides. First and foremost, by being comprised of thousands of individual organisms each with clearly defined functions within and among different castes, the health and propagation of a colony depends on the collective interactions among bees both inside and outside the hive. A single hive can contain 50,000 or more bees of which the vast majority are sexually undeveloped female workers; other castes include male drones, developing larvae and pupae (collectively referred to as brood), and a single reproducing queen. While the largest number of bees in the colony are typically represented by female workers who forage for food, younger worker bees are confined to the hive where they serve in various capacities (*e.g.*, nurse bees, mortuary bees, guard bees). Hence, the term "superorganism" has been devised to describe the interdependent structure and function of the honey bee colony (Tautz 2008). As a result, the translation of the loss of one or more individual bees into effects at the whole colony level depends on a number of factors. These factors include the nature of the interactions (including extensive communication mechanisms), the extent of loss of various hive functions, and the ability of individuals within the colony to compensate for lost or reduced functions served by these bees[3]. While the intent of this white paper is to focus on a risk assessment process for honey bees, the data provided through studies conducted on individual bees have utility in assessing the potential effects of pesticides on solitary bees which do not form colonies.

A second attribute important to risk assessment pertains to the vast differences in exposure potential to pesticides in terms of the magnitude, timing and duration, frequency and medium of exposure among and within different castes of bees. Pollen serves as the main protein source for bees, while plant nectar serves as the main source of carbohydrates (sugar). Foragers have the most direct contact with foliarly applied pesticides via spray droplets, inhalation, and contact with foliage. They are also highly exposed to pesticide residues via pollen and nectar used for food as well as plant resins (propolis) used to seal hives. In contrast, bees living within the hive (*e.g.*, nurse bees, mortuary bees, wax producing bees, queen bee, larvae) are exposed primarily through consumption and processing of pollen, nectar, wax,

[3] Loss of the queen is a notable exception which can lead to the loss of a colony. However, hives have the ability to respond to queen loss by forming a new queen. Furthermore, loss of older foragers can result in younger brood attending bees taking on foraging responsibilities to maintain adequate food supplies.

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honey and propolis. Nurse bees, for example, consume relatively high amounts of bee bread, which is a fermented mixture of pollen and honey, while developing brood consume protein rich secretions known as brood food (worker jelly) and royal jelly from paired glands (hypopharyngeal and mandibular glands) located in the frontal area of the head of young worker bees (nurse bees); nurse bees also distribute the jelly to nest mates through trophallactic (*i.e.*, exchanging secretions between bees) interactions (DeGrandi-Hoffman *et al*. 2010). The queen bee, on the other hand, consumes royal jelly throughout her life in the hive. Larvae that develop into workers are fed royal jelly early on in their development, after which time they are fed brood food. In winter, adult workers consume large amounts of stored honey as an energy source during periods where thermoregulation (via exercising flight muscles) is necessary.

A third important factor to consider in risk assessment is that the entire reproductive capacity of the colony is critically linked with the queen. Most species have large numbers of reproducing individuals within any given population, and thus the loss of one reproducing individual may be of little overall consequence to the population. In the case of honey bees, the queen is the only individual responsible for laying eggs, which can be as high as 1,200 per day. Thus, the health and overall fate of the queen becomes a critical component of the success of a colony.

Lastly, it is important to consider both the temporal and spatial scales on which honey bees may be exposed to pesticides. Workers usually forage within 1-2 km of the hive, but may reach distances of 7 km (5 miles) in some instances. Thus, the spatial scale of potential pesticide exposure can be much larger than typical sizes of fields planted in crops and may encompass multiple cropped and non-cropped areas at any given time. Temporally, honey bees may be exposed not just during or near pesticide application, but potentially over much longer time periods for highly persistent pesticides due to processing and storage of hive materials (*e.g.*, bee bread, honey, wax, and propolis) or through collecting exudates from extra-floral nectaries when plants are not in bloom.

1.4 International and Domestic Efforts to Refine Effects and Risk Assessment Methods for Bees

A number of efforts have been underway to improve and refine pesticide risk assessment methods for pollinators. The International Commission for Plant-Bee Relationships (ICP-BR), which is affiliated with the International Union of Biological Sciences, has been developing harmonized methods for testing the toxicity of pesticides to bees. The efforts of this organization inform decisions by regulatory authorities

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in Europe, *e.g.*, the European and Mediterranean Organization for Plant Protection (EPPO) as well as the Organization for Economic Cooperation and Development (OECD). The ICP-BR Bee Protection Group has regularly scheduled symposia which draw on expertise of government, industry and academia to inform scientific/risk assessment efforts aimed at bee protection. The proceedings of the 10th International Symposium of the ICP-BR Bee Protection Group, reported on efforts to revise the EPPO risk assessment scheme (Alix *et al*. 2009) for plant protection products (*i.e.*, pesticides) and the studies used to inform that process (Lewis *et al*. 2009).

In July 2009, EPA hosted a USDA-sponsored meeting on Pollinator Toxicity Testing. The meeting served as a forum for researchers to discuss current testing protocols for acute and chronic toxicity studies for honey bees and to identify uncertainties and limitations in those protocols. The goal of this forum was to consider refinements and expansion of current test designs toward more comprehensive and standardized protocols and increase consistency and reproducibility and enhance the utility of these studies in regulatory decision making (USDA 2009).

In January of 2011, the Society of Environmental Toxicology and Chemistry (SETAC) sponsored a global Pellston[4] workshop on pollinator risk assessment. The intent of the workshop was to bring together the best available science regarding pesticide exposure and effects assessment methods for honey bees and non-*Apis* bees and to further harmonize risk assessment approaches among North and South America, Europe, Australia, and Africa for a global improvement of the protection of insect pollinators in cropped areas. Work at the Pellston was organized to cover four primary components of evaluating risk: exposure assessment, laboratory effects assessment, semi-field and field effects assessment, and integrating exposure and effects data to estimate the likelihood and magnitude of potential adverse effects, *i.e.*, risk assessment. A fifth area of expertise was identified on non-*Apis* bees and was charged with determining the extent to which honey bee exposure, effects and risk assessments could be used

[4] The first Pellston Conference was held in 1977 to address the needs and means for assessing the hazards of chemicals to aquatic life. Since then, many conferences have been held to evaluate current and prospective environmental issues. Each has focused on a relevant environmental topic, and the proceedings of each have been published as a peer-reviewed or informal report. These documents have been widely distributed and are valued by environmental scientists, engineers, regulators, and managers because of their technical basis and their comprehensive, state-of-the-science reviews. The first four Pellston conferences were initiated before the Society of Environmental Toxicology and Chemistry (SETAC) was effectively functioning. Beginning with the 1982 conference, however, SETAC has been the primary organizer and SETAC members (on a volunteer basis) have been instrumental in planning, conducting, and disseminating conference results. Taken from: http://www.setac.org/node/104

to assess risk to non-*Apis* bees. An executive summary (Fischer and Moriarty 2011) of the workshop was published on-line by SETAC in September 2011 and the full proceedings will be published in late 2012.

The European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR) was asked to deliver a scientific opinion on the science behind the development of a risk assessment process for bees including both *Apis* and non-*Apis* bees (EFSA 2012). In the resulting opinion, EFSA identified specific protection goals based on an ecosystem services approach that included ensuring pollination services, production of honey bee hive products and biodiversity; these protection goals are the same as to those identified through the SETAC Pellston (Fischer and Moriarty 2011). The opinion examined potential routes of exposure to categories/castes of bees (*e.g.*, colony foragers and/or larvae versus solitary bees) and it examined the strengths and limitations of current toxicity testing methods along with recommendations on how to improve these methods. Recommendations included the need for an expanded pesticide toxicity testing including contact and inhalation studies with both adult and larvae and which include sublethal measurement endpoints as well as lethality. The EFSA also noted that few studies have been conducted thus far on non-*Apis* bees that consider endpoints such as fecundity, larval mortality rate, adult longevity and foraging behavior even though micro-colonies of bumble bees may be suited to measure lethal and sublethal effects from varying durations of exposure. This opinion is intended to serve as a basis for the development of a guidance document for regulatory authorities on assessing the risks of pesticides to pollinator insects. As noted earlier, the EFSA opinion has informed the process outlined in this white paper.

EPA, as well as PMRA, has engaged its partners of the OECD to find areas where cooperation would advance the science and/or the management around protecting pollinators. To that end, EPA and PMRA are working with (17) OECD countries on issues relating to pollinator protection. This effort has focused on collaboration and coordination in the areas of science and policy. In 2010, the OECD published the results of a survey of member countries on pollinator testing, mitigation and information management (OECD 2010). As a result of the survey, specific focus areas for additional work included:

1) advancing and harmonizing the science of risk assessment;
2) developing a compendium of risk management approaches;
3) developing tools to share incident information; and,
4) providing an index of research relevant to pollinator health and protection.

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EPA and PMRA have continued to work with OECD partners and have formed a Pesticide Effects on Insect Pollinators (PEIP) workgroup to address each of these focus areas.

1.5 Current Risk Assessment Process

1.5.1 Overview

In North America, both EPA and PMRA have developed a tiered system for assessing the effects of pesticides to non-target organisms. While the goal is to be protective of a broad number of beneficial terrestrial invertebrates, the regulatory agencies have relied on surrogate species to represent these assemblages (including insect pollinators). In this testing system, the honey bee serves as the surrogate for non-target insects and for insect pollinators; however, when there are non-*Apis* data to evaluate potential risks to non-target insects (*e.g.*, from the scientific literature), those data are considered. In the US, the Code of Federal Regulations (CFR) 40, Part 158 (abbreviated 40CFR158) defines the data required to support the registration of a pesticide, while in Canada data requirements are outlined in Directive DIR2003-01 (Health Canada 2003), which lists and defines all the Use Site Categories for conventional chemicals and refers to the data-code tables posted on the PMRA website (DACO tables; Health Canada 2012). Similar to other taxa for which data requirements exist, the data requirements in North America are tiered. Initially, studies are conducted on individual organisms under laboratory conditions where the environment can be readily controlled. Higher tier studies typically transition from the laboratory to field and focus on larger numbers of organisms under much less controlled conditions.

The first tier of testing in North America consists of an acute contact toxicity test, *i.e.*, OCSPP Guideline 850.3020 (USEPA 2012*a*) or OECD Guideline 214 (OECD 1998*a*) on adult honey bees that provides a median Lethal Dose (LD_{50}), *i.e.*, the lethal dose that causes death of 50% of the exposed organisms, along with any sublethal effects (*i.e.*, effects which do not result in direct mortality) that may have occurred as a result of chemical exposure. In addition to a contact toxicity test, an acute oral toxicity test is also required in Canada that provides information on the oral LD_{50} from a single dose of the test compound, along with any sublethal effects (OECD Guideline 213 (OECD 1998*b*). The acute contact and oral LD_{50} is assessed after 24 and 48 hours. Depending on the outcome of these toxicity tests, pesticides are classified as practically non-toxic (LD_{50}>11 μg a.i./bee), moderately toxic (LD_{50} is 2- 11 μg a.i./bee), or highly toxic to bees (LD_{50} <2 μg a.i./bee) on an acute exposure basis. If the acute contact LD_{50} is less than 11 μg active ingredient/bee, additional testing may be required in the form of a foliar residue study

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(OCSPP Guideline 850.3030; USEPA 2012*b*) to determine the duration over which field-weathered foliar residues remain toxic to honey bees. On a case-by-case basis, agencies may also require additional higher-tiered studies such as hive studies (published methods including Ooman *et al.*, 1992) and semi-field and field pollinator studies, *i.e.*, OCSPP Guideline 850.3040 (USEPA 2012*c*), OECD Guidance Document 75 (OECD 2007), and the EPPO Standards PP1/170 (OEPP/EPPO 2010) if the data from toxicity studies indicate potential chronic effects or adverse effects on colonies.

In the U.S. and Canada, risk assessment relies on multiple lines of evidence. While pesticide manufacturers are required to conduct studies in support of the registration of these products, regulatory authorities are not confined to these studies as the sole source of information regarding the potential for the use of a pesticide to result in adverse effects on the environment. Risk assessors make use of information reported in the open literature as well as incident reports. Depending on the level of detail of information contained in these alternate sources, the information may be used quantitatively, *i.e.*, to calculate point estimates of exposure and effect thresholds for use in calculating a risk quotient (RQ), or qualitatively (to characterize risk identified using RQs) to describe potential adverse effects resulting from the use of the compound and to develop appropriate label language to mitigate the potential effects.

1.5.2 Limitations

At this time, the major limitation to the current process has been the lack of a clear, comprehensive and quantitative process for evaluating pesticide exposure and subsequent risk to bees from different routes of exposure. Bees may be exposed to pesticides through multiple routes (*e.g.*, direct contact with pesticide residues and/or ingestion of contaminated pollen/nectar). Historically, EPA and PMRA have not had a means of consistently estimating exposure to bees through either direct contact or via ingestion of food with pesticide residues, nor have data been routinely available on measured pesticide residues in pollen, nectar and honey. While residue data have been collected as part of the battery of tests used to establish pesticide tolerances and/or to determine potential worker exposure, these data have not typically included matrices (*i.e.*, pollen, nectar and honey), which would be informative to determining potential exposure to bees.

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The current battery of toxicity tests identified in the 40CFR158.630 and DIR2003-01 for evaluating the potential effects of pesticides on honey bees is also limited. The laboratory-based studies provide information on only young adult worker bees with a short duration of exposure and do not provide information on other life stages, *i.e.*, developing larvae/pupae (brood) or caste members (i.e., queens, and drones. Although acute oral toxicity data with adult worker bees are routinely considered by PMRA, these data are not required by EPA; however, these data are now more commonly available and are considered by EPA for risk assessment if available. As mentioned earlier, the laboratory-based studies focus on individual bees, yet bees must be considered within the context of the larger colony (hive) in which they live. Although current test guidelines stipulate that sublethal effects must be reported in the laboratory-based studies, the primary focus of these studies is frank mortality; therefore, the exposure concentrations used in the studies may mask sublethal effects. Open literature and registrant-submitted studies have continued to identify an increasing number of sublethal measurement endpoints; however, there is uncertainty as to how many of these measurement endpoints are related to regulatory assessment endpoints of impaired growth, survival and reproduction of the hive. Until sufficient linkages have been developed which enable risk assessors to extrapolate sublethal measurement endpoints to assessment endpoints, their utility in a regulatory context may continue to be limited.

For those chemicals where there is considerable uncertainty regarding the potential for adverse effects on the entire colony, brood/hive studies, semi-field, or field pollinator studies may be required. Although field pollinator studies are meant to represent more realistic conditions, the execution and interpretation of field-based studies with freely foraging bees can be challenging as it can be difficult to ensure that test bees will forage in a particular area given that they can forage over 7-km from their colonies depending on the availability of forage. While honey bees can exhibit some crop fidelity, *i.e.*, foraging on a specific crop, the extent to which this occurs is uncertain and is also difficult to control in free-foraging bees. In an attempt to account for these factors, protocols for these studies have varied widely and these studies have frequently been confounded by the presence of other chemicals within the colony or the effective forage area of the bees. Although field studies are intended to be more representative of actual conditions of use and exposure, other factors influencing the colony are difficult to consistently control; therefore, disease, parasites, nutrition, and bee management practices can be confounding effects on these studies.

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As noted previously, the current process within EPA is to rely on the battery of toxicity tests to determine potential hazards to honey bees. Historically, EPA has qualitatively assessed potential hazard and not risk since toxicity estimates alone were used. Estimates of potential exposure levels have not been generated and therefore, the traditional quotient-based risk characterization approach used for other taxa has not been established for honey bees. In addition to using the potential hazard for determining label language, PMRA has determined a risk quotient based on the method described by Atkins *et al.* 1981 which is discussed later in this paper.

The EPA has developed interim guidance (USEPA 2011) to ensure consistency in requests for pollinator exposure and toxicity data to inform risk assessments and decision. In case-by-case situations, EPA is asking for additional non-guideline data to reduce uncertainties related to potential exposure and effects. These special studies include honey bee acute oral toxicity tests, acute larval toxicity tests, semi-field toxicity tests with honey bee colonies confined to tents/enclosures or fed with an artificial diet, and full field studies. Additionally, to more realistically characterize exposure, residue studies in pollen and nectar are considered. This interim guidance also reflects the PMRA practice of requesting additional pollinator data (*e.g.*, field pollinator studies) to inform risk assessments and decisions.

Although toxicity testing is routinely required to understand the potential effects of pesticides on non-target arthropods, *e.g.*, honey bee toxicity data, and the results from these studies have been used to determine appropriate label language to reduce potential hazards, an overall decision framework for quantifying risks from pesticides to bees has been lacking. The interim guidance provided to EPA risk assessors on both toxicity and exposure data requirements was intended to assist in characterizing potential hazards to bees as opposed to quantifying potential risks. Only through the development of a reliable means of quantifying exposure using either screening-level models and/or more refined measures of actual residues could a process consistent with that used for other taxa be developed for quantifying risk and for evaluating how mitigation measures may affect risk estimates. Such a process would also enable the development of a decision framework for better allocating resources to those chemicals which may require more refined assessments to determine the spatial and temporal scope of their potential effects on bees. The development of a risk assessment process for bees is the focus of this white paper.

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1.6 International Risk Assessment Process

1.6.1 Overview

In the European Union (EU), risk to honey bees from exposure to pesticides (referred to in the EU as "plant protection products") is assessed according to the EPPO standards PP 3/10 (2) (OEPP/EPPO) and includes a tiered progression of testing described by the guideline No. 170 (OECA/EPPO 2010). This guideline describes lab tests, semi-field (cage/tunnel) tests, and field tests for evaluating the lethal and sub-lethal effects of agrochemical products on honey bees. The testing approach in the EU is similar to that of the EPA and Canada in that it consists of a tiered approach. In contrast to EPA, the EU and Canada require the acute oral toxicity (LD_{50}) on adult workers in addition to the acute contact toxicity. In the EU, it is also standard practice to conduct both acute oral and contact laboratory LD_{50} studies on formulated end-use products as well as the active ingredient (a.i.), and Canada also regularly receives and reviews these acute oral and contact studies on formulated end-use products. EPA also receives these studies when it is engaged in a global review with its European partners. The EU relies on a three-tier process, with initial studies conducted in the laboratory, followed by semi-field studies, and finally field studies. The trigger criterion for assessing potential risk to adult bees as a result of foliar spray treatments and for moving from Tier I (laboratory tests) to Tier II (semi-field tests) and/or Tier III (field tests) is the Hazard Quotient (HQ), where HQ is the ratio of the field application rate in grams of active ingredient per hectare (g a.i./ha) to the oral or contact LD_{50} (whichever is lower) expressed as micrograms of active ingredient per bee (μg a.i./bee). Comprehensive reviews of pesticide bee kill incidents show that bee kills have never been observed when the HQ<50. Therefore, a HQ of 50 is used as a decision criteria to determine whether risk of acute lethality can be precluded (*i.e.*, when the HQ<50) or whether it requires further study (*i.e.*, when the HQ>50 indicating the need for higher tier testing). Higher tier tests like semi-field and field tests under controlled exposure conditions typically measure effects on adult bee behavior, queen performance, brood development, and overall colony performance.

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1.6.2 Limitations

Although the EU's (lower tier) testing and risk assessment scheme for foliar application is more detailed than the one used in North America in that it defines measures of exposure as well as effects used in estimating risk, the EU is looking to expand its risk assessment capability even further. The EU believes that more standardized tests for measuring effects of plant protection products to brood and for measuring sublethal effects to honey bees are needed as an intermediate step. Efforts have been made in Europe to develop more standardized procedures for measuring effects to brood. In addition, the determination of the acute LD_{50} and associated sublethal effects for oral and contact exposures at 24 and 48 hours versus some other length of exposure is somewhat arbitrary and needs to be further vetted. In the risk assessment scheme of systemic products, due to their exposure scenario, it may be appropriate to develop an ED_{50} or EC_{50} (median effect dose or concentration for 50% of the organisms tested) for chronic exposure similar to the requirements in assessing risk to other taxa (*e.g.*, aquatic invertebrates) when study methods become available.

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2 Proposed Risk Assessment Process

Although the role of pesticides in pollinator declines has not been well established, global experts from different disciplines (*e.g.*, chemistry, ecotoxicology and entomology) and across various sectors (*e.g.*, government, academia, non-governmental regulators and industry) agree on the need to advance the science to better assess potential exposure, hazard and risk to honey bees and other insect pollinators from pesticides used in agriculture. The need to advance the science has been accentuated with the development of newer pesticides which are systemically distributed in plants following applications to soil, seeds and tree trunks. For these applications, pesticide exposure through the diet is expected to be the major route of exposure. This is in contrast to exposures of bees via both contact and diet that arise from more traditional pesticides that are applied via foliar spray. The proposed process which the SAP is being asked to consider reflects a synthesis of domestic and international efforts to develop a means for quantifying the potential risks of pesticides to bees for use by North American pesticide regulatory agencies (EPA, CalDPR and PMRA). Therefore, the recent work by the global SETAC Pellston conference on pollinator risk assessment (Fischer and Moriarty 2011), the scientific opinion by EFSA (EFSA 2012), and the efforts of EPPO, ICP-BR, and the OECD Pesticide Effects on Insect Pollinators work group have been used to inform the proposed process.

As with risk assessments for other taxa, the proposed risk assessment methods described in this document make use of a surrogate species. The ecological risk assessment process consists of a series of steps which are intended to be iterative. In general, the risk assessment process for evaluating pesticides entering the market or those undergoing re-evaluation as in the US and Canada, consists of problem formulation, analysis and risk characterization (USEPA 1998). This generic process is depicted in **Figure 1**. In problem formulation, risk assessors define protection goals based on discussion with risk managers, identify assessment and measurement endpoints, prepare a conceptual model and develop an analysis plan for the risk assessment. Based on the conceptual model and its associated risk hypothesis, the analysis plan articulates how the risk hypothesis will be tested.

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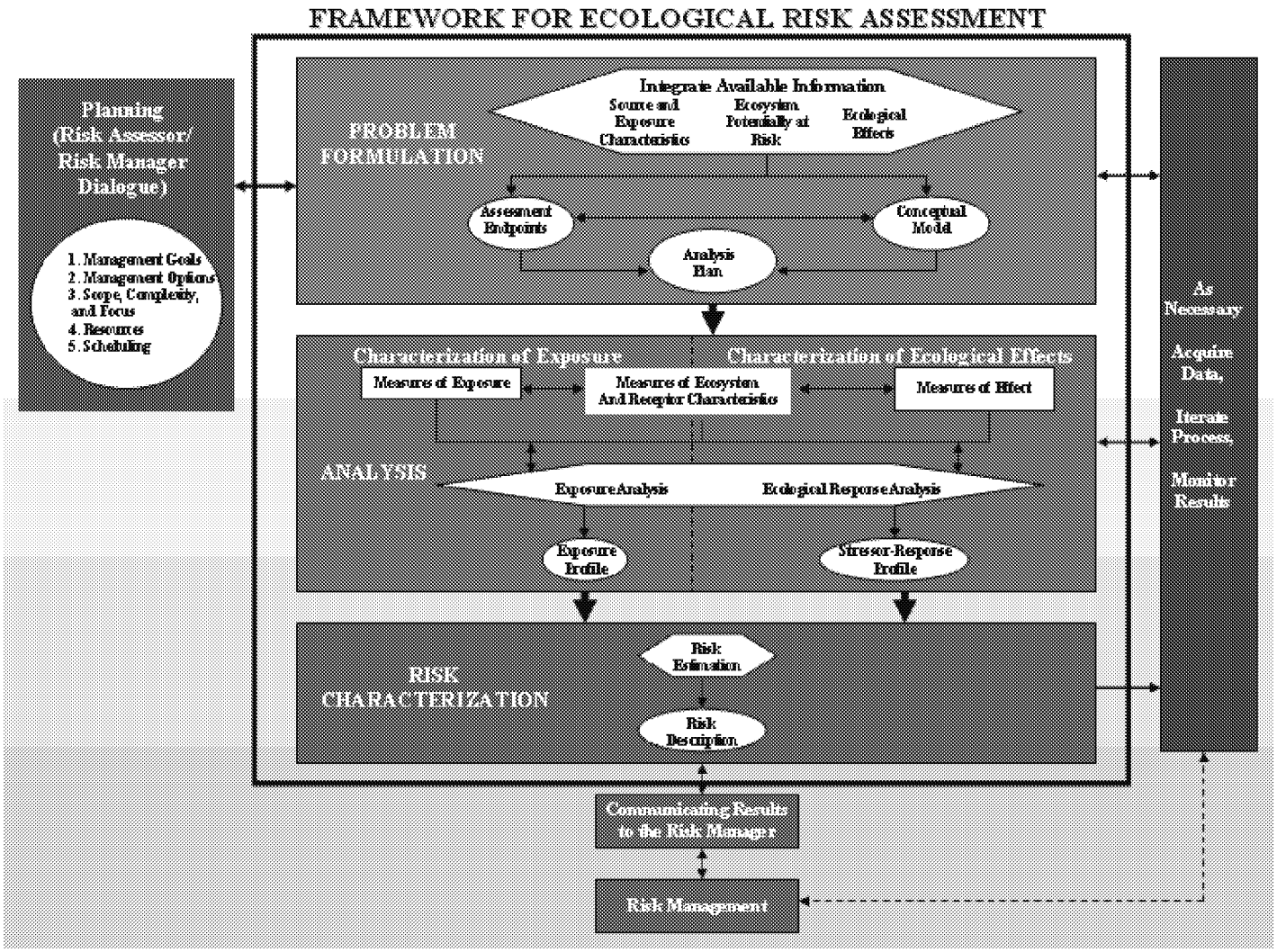

Figure 1. Diagram of the iterative risk assessment process consisting of three phases, *i.e.*, Problem Formulation, Analysis and Risk Characterization.

During the Analysis step, available measures of exposure and of effects are evaluated and the movement of a stressor (*i.e.*, pesticide) through the environment is characterized based on its intended use pattern(s); this is frequently termed the exposure characterization or exposure profile. Similarly, the potential effects of a chemical are characterized in what is frequently termed the effects assessment. Once effects and exposure are characterized and estimates and/or measures are available, the risk assessment proceeds to the risk characterization step. Typically, the risk characterization consists of two steps, *i.e.*, risk estimation and risk description. In the risk estimation step, the measures of exposure and measures of effect are integrated to develop risk estimates. These estimates may be based on point estimates of exposure, *e.g.*, maximum estimated exposure values based on the maximum application

rate for a particular use, and point estimates of effect, *e.g.* the acute median lethal concentration to 50% of the species tested (LD_{50}). Alternatively, risk quotients could be based on distribution-based estimates of both exposure (*e.g.*, maximum residue concentrations in pollen from several field monitoring studies based on maximum application rate for a particular use), and effects (*e.g.*, species sensitivity distribution using LD_{50} values). Regardless of whether point and/or distribution-based estimates are used, the integration of exposure and effects data is typically expressed as a ratio (quotient) of estimated exposure and the effect endpoint; this ratio is considered the "risk estimate". Deterministic estimates of risk, based on point estimates of exposure and effect endpoints, do not typically provide information on the magnitude and likelihood of adverse effects; whereas distribution-based estimates of both exposure and effects that lead to joint probability distributions are frequently used to estimate both the likelihood (probability) and magnitude of an adverse effect. The decision to move from point-estimate based approaches to distribution-based approaches that may also be spatially and temporally specific are determined by the availability of data, the extent of variability associated with exposure and effect estimates, and by the need for additional information to support a risk management decision.

The second part of risk characterization is the risk description where quantitative estimates of risk are further described qualitatively using other data as well. Multiple lines of evidence are used to describe what is known about potential adverse effects resulting from the use of a pesticide. Risk descriptions include additional information about the variability associated with what has been measured or estimates/assumed along with associated uncertainties. The effects of potential mitigation measures and uncertainties on risk assessments may also be described. Therefore, although the risk description portion of the characterization is described as “qualitative”, it often includes various quantitative elements (*e.g.*, point estimates of exposure or toxicity endpoints. The risk characterization is intended to provide a transparent, clear, concise and reasonable synthesis of the overall conclusions (USEPA 2000).

Although the risk assessment process is depicted as three distinct steps, each step is intended to be iterative. As more information (data) becomes available, the process can evolve if needed to further refine risk management decision making. The risk assessment process is, therefore, intended to take advantage of multiple lines of evidence, and the problem formulation with its conceptual model and risk hypothesis may change as more information becomes available. A critical component to this iterative process is frequent and clear communication with risk managers to ensure that protection goals are

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adequately accounted for and that the effects of uncertainties and potential mitigation measures on risk estimates are evaluated.

Consistent with the iterative nature of the risk assessment process, regulatory agencies typically rely on a tiered process for conducting ecological risk assessments. The preliminary or screening-level (Tier I) assessments are intended to determine whether potential risks exist while higher-tier assessments (Tiers II and III) attempt to refine and/or characterize risk estimates to determine the conditions of risk occurrence and, when relevant, to identify spatially- and temporally-specific risks. The tiered risk assessment process enables regulatory agencies to identify those chemicals for which higher levels of resources should be devoted to support a more refined approach.

In a tiered, iterative process, the risk assessor and risk manager work together to evaluate the results of each step and determine whether mitigation measures can be developed to reduce exposure. Where such measures are implemented, the need for additional refinements is eliminated.

The proposed quantitative risk assessment process for pollinators adheres to the basic elements described in the preceding sections and is, therefore, consistent with the process used for other taxa. As with other taxa, if risks are identified in the screening-level risk assessment and/or if the risk manager requires additional information to support a regulatory decision, the risk assessment may be further refined through more detailed analyses of exposure and/or effects. With every iteration, estimates of exposure and effects are intended to produce more realistic values that are reflective of what may occur under actual use conditions of the pesticide.

Consistent with the proposed approach vetted at the SETAC Pellston workshop (Fischer and Moriarty 2011) and the EU (Alix *et al*. 2009), there is recognition that the potential risk to bees from the use of pesticides may differ by use or by chemical. For example, pesticides applied via foliar spray may reach bees through contact and through the diet, whereas dietary exposure would be the major route expected for pesticides that are systemic and applied to the soil, seed or tree trunks. For seed treatments of systemic pesticides, pesticide exposures are expected to occur through the diet. In addition, it is recognized based on incident data from Germany (Pistorius *et al.* 2009, Forster 2009) and through studies reported in the open literature (Krupke *et al.* 2012, Tapparo *et al.* 2012) that seed treatments can result in the formation of dusts from abraded treated seed coat during planting and that

these contaminated dusts can serve as a source of contact exposure. Risk assessments may differ on a chemical-by-chemical basis as well. For instance, in cases where a pesticide has toxic degradates, additional data may be required for those degradates.

2.1 Decision Tree

Figure 2 illustrates the proposed decision-making process for assessing risks to honey bees associated with foliar spray applications of pesticides (*e.g.*, via ground and aerial methods) while **Figure 3** illustrates the process for soil and seed treatments (*e.g.*, soil drench, seed treatments). These decision-making frameworks are introduced to provide context to the detailed discussions of each of these risk assessment and decision steps described in the ensuing sections. The overall proposed approach is a tiered process whereby risks are first assessed using simple and conservative exposure screening models to generate estimated environmental concentrations (EECs) (**Boxes 3a**, **3b** and **3c** of **Figures 2** and **3**) coupled with toxicity estimates derived from laboratory studies (Tier I) to calculate risk quotients (RQs) (**Boxes 4a**, **4b** and **4c** of **Figures 2** and **3**). Results from the Tier I risk assessment process are expected to be reasonably conservative such that the likelihood of a false negative is low (*i.e.*, the chance that no risk is indicated but risks actually occur), while at the same time ensuring that the likelihood of a false positive (*i.e.*, the chance that risk is indicated when none actually exists) is not unacceptably high. For example, the initial exposure estimates used in Tier I are generally not chemical-specific, but rather reflect upper-bound estimates that would encompass exposures across all relevant pesticide uses. If risks are identified in Tier I (*i.e.*, where risk estimates exceed levels of concern; **Box 5** of **Figures 2** and **3**), additional data may be used to refine the results, such as using estimates of exposure derived from available magnitude of residue or other commonly submitted studies (**Box 6** of **Figures 2** and **3**).

If risks are still identified after refinement with available data (**Box 7** of **Figures 2** and **3**), then appropriate risk mitigation options would be identified and further evaluated for their impact on risk estimates (**Box 8** of **Figures 2** and **3**). Alternatively (or in addition), a higher tier assessment may be necessary (Tier II) and studies providing refined estimates of exposure (*e.g.*, field studies quantifying residues in pollen and nectar; **Box 9a** of **Figures 2** and **3**) and effects at the colony level (*e.g.*, semi-field tunnel studies or field-level feeding studies; **Box 9b** of **Figures 2** and **3**) may be requested. Measured residues in pollen and nectar (**Box 9a** of **Figures 2** and **3**) from these studies may be used to refine risk estimates from Tier I (**Box 6** of **Figures 2** and **3**) and/or for qualitatively evaluating risk at the colony level

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associated with pesticide applications (**Box 10** of **Figures 2** and **3**). They may also be used to identify more targeted risk mitigation options than those that could be identified based on Tier I risk estimates.

Although not specifically depicted in **Figure 2** for foliar applications, data from the toxicity of residues on foliage study are used qualitatively to characterize the length of time that residues remain toxic to bees. The results of the guideline study may result in precautionary label statements similar to those discussed in the EPA Label Review Manual (USEPA 2012) or in guidance documents intended to reduce the potential effects of pesticides on bees (e.g., Riedl *et al.* 2006).

If available risk mitigation options (**Box 11** of **Figures 2** and **3**) do not provide for an acceptable reduction in risk, proceeding to Tier III (**Box 12** of **Figures 2** and **3**) may be necessary to resolve specific uncertainties identified from Tiers I and II for the proposed uses of the pesticide. For example, effects on the ability of colonies to successfully emerge in the spring (*e.g.*, produce sufficient brood and adult bees after over-wintering) may be a concern for some pesticides/uses which are not typically addressed in earlier tiers.

The risk assessment process depicted in **Figures 2** and **3** is intended to be iterative and to rely on multiple lines of evidence to further refine and characterize potential risk. At a screening level, risk to individual bees is quantified through the use of RQ values. Where RQ values exceed the LOC, more refined estimates of exposure may be used to re-evaluate RQ values for individual bees based on laboratory toxicity estimates. Where RQs still exceed LOCs, higher tier semi-field and full-field studies may be required to determine whether effects observed under highly controlled conditions extend to the whole colony under increasingly realistic exposure conditions. As depicted in **Figures 2** and **3**, if the multiple lines of evidence indicate that unacceptable effects on survival, growth or reproduction of the colony are not likely, then a presumption of minimal risk can be supported. Alternatively, there may be situations where colony-level effects may be likely, given the proposed use or known mode of action of a compound. In this case, a presumption of minimal risk cannot be supported, and risk assessors should attempt to characterize the nature and possible magnitude and duration of the effect. This characterization should include a discussion of uncertainties which limit the extent to which the possible magnitude and duration can be estimated. Also, the risk characterization should include any potential mitigation options for minimizing risk to bees from the proposed use of a pesticide.

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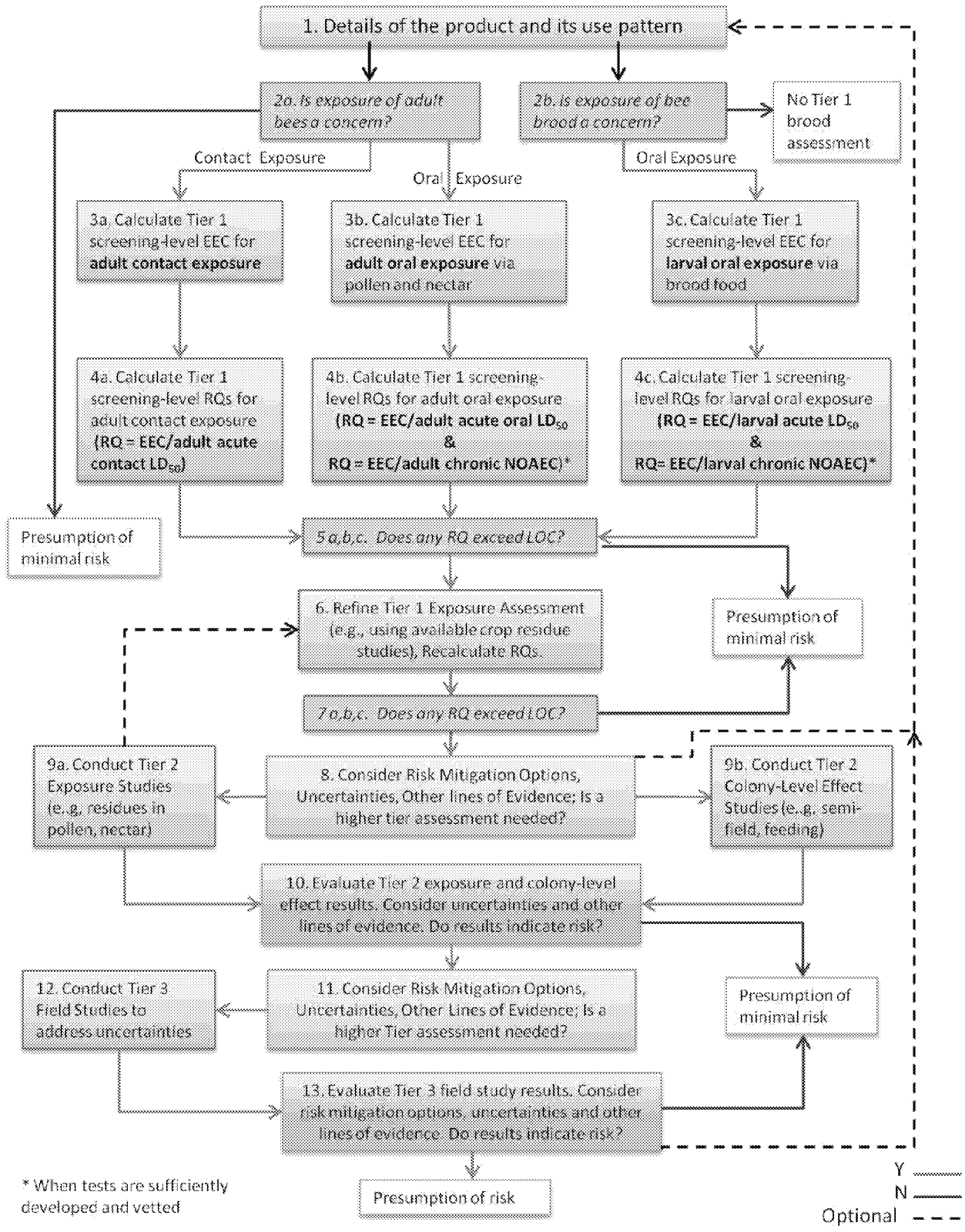

Figure 2. Proposed Tiered Approach for Assessing Risk to Honey Bees from Foliar Spray Applications.

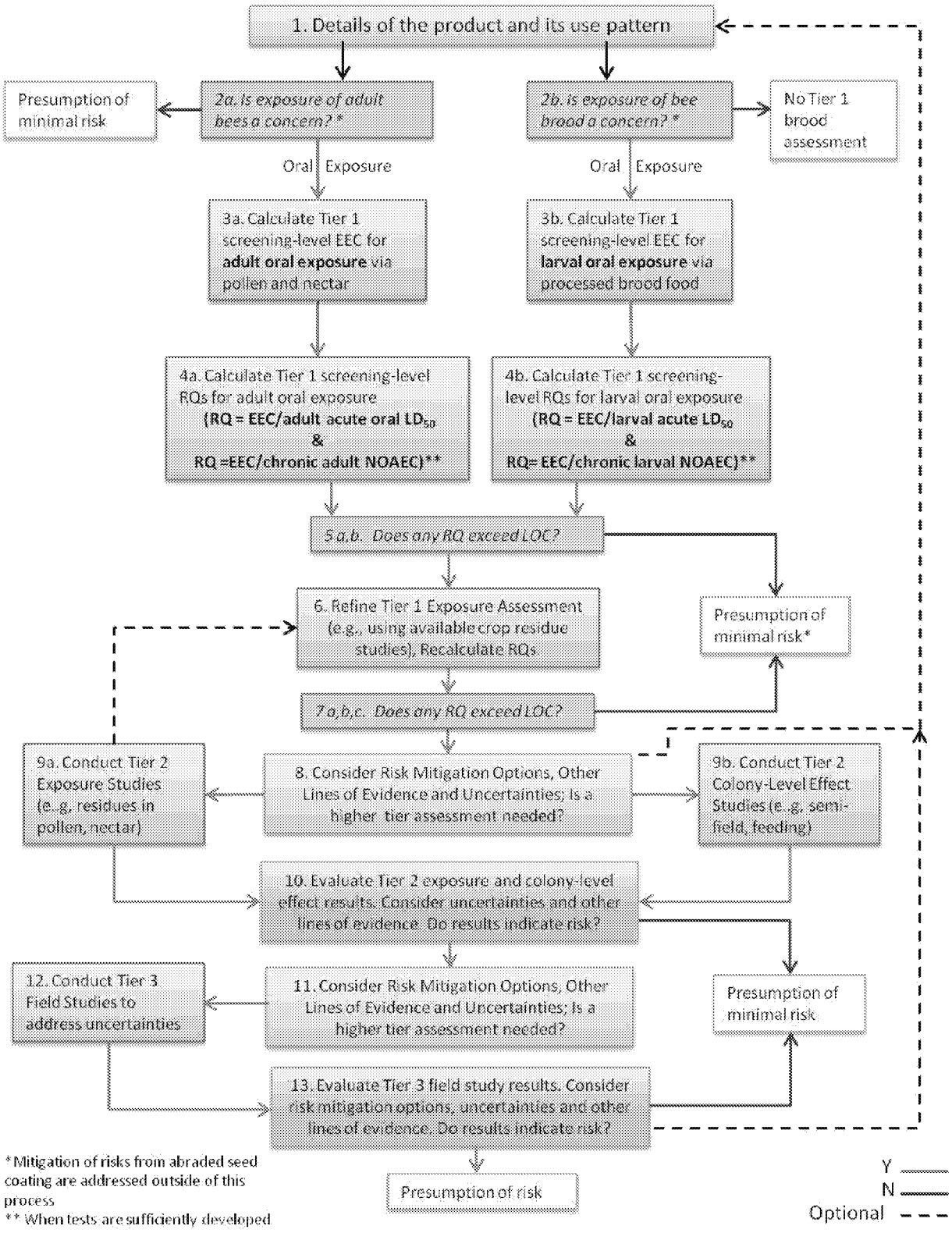

Figure 3. Proposed Tiered Approach for Assessing Risk to Honey Bees from Soil/Seed Treatments.

In the following section, the problem formulation of the risk assessment is discussed in greater detail as it relates to assessing risk to bees. As indicated earlier, problem formulation represents the first step of risk assessment where management goals are articulated along with the assessment and measurement endpoints used to evaluate whether those goals are affected. The problem formulation also identifies conceptual models and risk hypotheses which will be evaluated. Conceptual models serve as a graphic representation of the structure of the risk assessment. These models specify the stressor, *i.e.*, the pesticide applied to a specific site, its source (*e.g.*, whether the chemical is applied via foliar spray or seed/soil treatments), the exposure media (*e.g.*, whether exposure is likely through consumption of contaminated pollen and nectar versus residues on the plant's surface), biological receptors (*e.g.*, individual adult bees, larvae, pupae, colonies), and the attribute change (*e.g.*, reduced survival, decreased reproduction of the hive). Multiple conceptual models are depicted since multiple routes of exposure are possible.

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2.2 Problem Formulation

Problem formulation serves as the first step of a risk assessment and it provides the foundation for the entire ecological risk assessment. A problem formulation is intended to identify the objective of the risk assessment and is intended to produce three products: (1) assessment and measurement endpoints that adequately reflect management goals and the ecosystem they represent, (2) conceptual models that describe key relationships between a stressor (*i.e.*, pesticide) and assessment endpoint or between several stressors and assessment endpoints, and (3) an analysis plan (USEPA 1998). In the following sections, each of these products is discussed in greater detail relative to evaluating the potential risks of pesticides to bees.

2.2.1 Management Goals and Assessment Endpoints

The mission of OPP is to protect human health and the environment from potential risks from pesticides and toxic substances based on a strong foundation of science, transparency and the rule of law (USEPA 2010). As identified in its strategic plan in its efforts to ensuring chemical safety, EPA is leveraging available expertise, information and resources by collaborating with other countries, federal agencies, states, tribes and the public to improve chemical safety. The intent of this effort is to:

- control risks of new chemicals before they are introduced into commerce;
- evaluate chemicals already in use;
- develop and implement regulatory and other actions to eliminate or reduce identified chemical risks; and,
- make public the data necessary to assess chemical safety to the extent allowed by law.

Consistent with the Agency's mission and its 5-year strategic plan, EPA is collaborating with PMRA and with the CalDPR while taking advantage of work conducted in the EU through the EFSA and through research conducted by government, academic and industry (*e.g.*, ICP-BR and the SETAC global Pellston Workshop) both domestically and internationally to understand and incorporate state of the art methods for evaluating potential risks of pesticides to bees.

Ecological risk assessments are typically developed within a risk management context to evaluate human-induced changes that are considered undesirable (USEPA 1998). Changes often considered

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undesirable are those that alter important structural or functional characteristics or components of ecosystems; therefore, an evaluation of adverse effects may include consideration of the type, intensity, and scale of the effect as well as the potential for recovery. However, the acceptability of adverse effects is determined by risk managers after consideration of multiple factors which are used to assess the risks and benefits of a pesticide.

Risk assessment is intended to provide a basis for comparing, ranking and prioritizing risks such that the results can be used in cost-benefit and cost-effectiveness analyses which in turn offer additional opportunities to evaluate alternative management options. Therefore, it is important that risk assessments consider management goals and objectives as well as scientific issues when developing assessment endpoints and conceptual models during problem formulation. Depending upon available data and risk management goals, risk assessments may be based on qualitative descriptions of the likelihood of adverse effects or quantitative probabilities of the occurrence of adverse effects.

Management goals are statements about the desired condition of ecological values of concern. With respect to pesticides, management goals are in part defined by the laws which provide the statutory authority to regulate pesticides. Under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA[5]), EPA must ensure that the use of a pesticide does not cause unreasonable adverse effects on the environment. FIFRA defines "unreasonable adverse effects on the environment" to mean (Cornell Law School 2012):

> 1. any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or
> 2. a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under FIFRA section 346a of title 21. The Administrator shall consider the risks and benefits of public health pesticides separate from the risks and benefits of other pesticides. In weighing any regulatory action concerning a public health pesticide under this subchapter, the Administrator shall weigh any risks of the pesticide against the health risks such as the diseases transmitted by the vector to be controlled by the pesticide.

Therefore, under the FIFRA statute, EPA is required to consider the benefits as well as the risks associated with the use of any pesticide. In evaluating potential risks to non-target organisms, EPA examines the potential effects of pesticides on growth, reproduction and survival since these broad categories of effects are known to impact populations and communities.

[5] Summary of the Federal Insecticide, Fungicide, and Rodenticide Act http://www.epa.gov/lawsregs/laws/fifra.htmlSm

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To address this statutory standard, the Agency has developed regulations and guidance which specify measures or actions that must (or must not) be taken, rather than establishing a value-based management goal or desired state. This guidance provides a framework for the scope, focus and conduct of a risk assessment. Risk assessments are typically designed to determine if a pre-established decision criterion is exceeded. These criteria often contain inherent assumptions about exposure and conditions (*e.g.*, acute contact exposure) which must be clearly articulated to ensure that management issues are addressed. As will be discussed later in this paper, the decision criteria are used in a tiered risk assessment framework to determine how extensive an assessment should be, *i.e.*, whether additional refinements may be necessary. In higher tiers, other factors beyond decision criteria may be considered since the risk management question changes from yes/no questions at the Tier 1 level to questions of spatial and temporal relevance, duration, frequency, and magnitude of adverse effect at the Tier II and III levels.

Management or protection goals therefore reflect the statutory and scientific objectives of a regulatory agency. These goals then inform decisions about the type of data which are considered in terms of both exposure and effects and they provide a basis for specific criteria which are applied to determine whether additional refinements are needed and/or whether mitigation should be considered. Consistent with the results of the SETAC Pellston workshop (Fischer and Moriarty 2011), the specific protection goals proposed in this white paper include:

- protection of pollination services provided by bees;
- protection of honey production and other hive products; and,
- protection of pollinator biodiversity in terms of an adequate number and diversity of species that contribute to the health of the environment.

Notably, the recent scientific opinion by EFSA on the science behind the development of a risk assessment of plant protection products on bees (EFSA 2012) also identified pollination, hive products (relevant for honey bees) and biodiversity as relevant ecosystem services and values to protect.

Once management/protection goals are identified, specific assessment and measurement endpoints are developed. As defined in the EPA guidelines (USEPA 1998), assessment endpoints are explicit expressions of the actual environmental values that are to be protected and they are operationally defined by an ecological entity and its attributes. The ability of the assessment endpoints to support risk management decisions is dictated by whether they are measurable ecosystem characteristics that can

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adequately represent management goals. EPA has defined three criteria for determining the selection of assessment endpoints; these include:

- ecological relevance,
- susceptibility to known or potential stressors, and
- relevance to management goals (USEPA 1998).

Table 1 provides protection goals and examples of relevant assessment endpoints for bees that were discussed at the SETAC Pellston (Fischer and Moriarty 2011) and are consistent with those that underlie the approach proposed in this white paper. In addition to assessment endpoints, it is important to identify specific endpoints which will be measured in toxicity tests; these measurement endpoints must have clear linkages to assessment endpoints if they are to be used quantitatively in an assessment.

Table 1. Proposed protection goals and examples of associated assessment and measurement (population and individual) endpoints for bees.

Protection Goal	Assessment Endpoints	Measurement Endpoints (Population level and higher)	Measurement Endpoints (Individual Level)
1. Provision of Pollination Services	Population size and stability of managed bees	Colony strength and survival	Individual worker survival Queen fecundity Brood size Worker bee longevity
2. Production of Hive Products	Quantity and quality of hive products	Quantity and quality of hive products; residue levels on honey/wax	Individual worker survival Queen fecundity Brood success
3. Contribution to Pollinator Biodiversity	Species richness[1] and abundance	Colony strength and survival Species richness and abundance[1]	Individual worker survival Brood success Queen fecundity

[1]Use of honey bees as a surrogate for other insect pollinators has limitations; however, it is assumed that as with all surrogates, data on individual organisms as well as colony-level data would provide some relevant information on the potential effects of a pesticide on both solitary bees as well as eusocial taxa. In addition, protection of honey bees would contribute to pollinator diversity indirectly by preserving the pollination and propagation of the many plants species pollinated by honey bees, which also serve as food sources for other pollinating insects.

There are an increasing number of measurement endpoints reported in *Apis* and non-*Apis* bee toxicity studies which may be conceptually relevant to the proposed assessment endpoints, but for which quantitative relationships to assessment endpoints (*e.g.*, colony strength and survival) have not been developed. Although such endpoints may not be used quantitatively to estimate potential effects on assessment endpoints, they can be used qualitatively to characterize potential effects which may not have clearly established relationships to colony-level effects and for explaining mechanisms by which colony-level effects might occur. These effects are frequently referred to as sublethal effects and are discussed in greater detail in the Tier I effects section of this white paper. As part of those discussions, the concept of adverse outcome pathways (AOPs) is introduced. Although regulatory authorities have

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developed standardized suites of *in vivo* studies intended to provide endpoints which can be directly related to assessment endpoints, studies reported in open literature contain a broad array of endpoints which span multiple levels of biological organization. These include alterations to biochemical pathways (molecular changes), effects on feeding behavior (*e.g.*, proboscis extension reflex), and alterations in immune response of individual bees; however, no information may be available from these studies on subsequent effects on survival, growth or reproduction and frequently assumptions are made regarding potential relationships. There is also an increased demand to reduce the number of animals used in testing and to make greater use of the broader suite of effects particularly as newer measurement technologies (*e.g.*, genomics, proteomics), computational tools (*e.g.*, quantitative structure activity relationships), and an understanding of the underlying molecular pathways evolve. Consistent with the recommendations of the National Academies of Science on toxicity testing in the 21st Century (National Research Council 2007), EPA is committed to making greater use of these evolving tools once there are sufficient data to translate this information into endpoints meaningful to ecological risk assessments (*i.e.*, effects on survival, development and reproduction) which in turn affect populations. The AOP concept outlined by Ankley *et al.* 2009 represents a conceptual construct which portrays existing knowledge concerning the linkages between a direct molecular initiating event and other key events which lead to an adverse outcome at a biological level that is relevant to risk assessment. Establishing the necessary linkages which will enable the use of measurement endpoints from various levels of biological organization to predict ecologically relevant effects (adverse outcomes) is an area which will continue to evolve as more data become available.

Therefore, the proposed assessment endpoints used to support efforts to evaluate potential risk to bees (some of which are listed in **Table 1**) include impaired survival of individual bees (*e.g.*, acute and chronic mortality of adult and larval bees), survival of the colony, development (*e.g.*, relative number of different brood stages), and reproduction (*e.g.*, fecundity of queen). These are consistent with the assessment endpoints used to evaluate risks to other taxa. These assessment endpoints are also consistent with the protection goals identified earlier. Measurement endpoints used to reflect these assessment endpoints will be impaired individual or colony survival following acute exposure and impaired individual or colony survival, development (growth), or reproduction following chronic exposure.

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2.2.2 Conceptual Model

An important element of ecological risk assessment is identifying the exposure pathway(s) by which a pesticide moves in the environment from its source to an ecological receptor (in this case, honey bee colonies). For an ecological pathway to be complete, it must have a source, a release mechanism, an environmental transport medium, a point of exposure for ecological receptors, and a feasible route of exposure.

A conceptual model provides a written description and visual representation of the predicted relationships between the stressor (pesticide), potential routes of exposure, and the predicted effects for the assessment endpoint. A conceptual model consists of two interrelated components: a conceptual diagram and risk hypotheses (USEPA, 1998). The conceptual model is developed from information collected and evaluated during the problem formulation process. Risk hypotheses are developed that reflect specific assumptions about potential adverse effects (*i.e.*, changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (USEPA 1998).

To illustrate the current understanding of the relationship between pesticide releases to the environment and changes in the proposed assessment endpoints described earlier, a series of *generic* conceptual diagrams and risk hypotheses are presented in this section. These diagrams were informed by two recent reviews related to pollinator risk assessment (Fischer and Moriarty 2011, EFSA 2012). The intent of these generic conceptual diagrams and risk hypotheses is two-fold: first, to serve as the conceptual basis of the proposed risk assessment process for honey bees presented in this white paper and second, to provide a basis for conceptual models to be used in future EPA and PMRA pesticide risk assessments. There are many factors that determine the exposure of honey bees to a pesticide, including methods of application, application rate, crop (some crops are not attractive to bees), and whether a pesticide is systemic are particularly useful in distinguishing the types of exposure pathways that must be considered in a risk assessment. As such, the example conceptual models provided below are organized according to application method and the systemic nature of pesticide uptake by plants. Because pesticide exposure pathways can differ greatly among different groups of honey bees, separate consideration is given to specific differences that may be described by age (*i.e.*, larvae or adults), task of adult worker (*e.g.*, foragers or nurse bees) and castes (*i.e.*, worker, queen, or drone). Importantly, these

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conceptual models are considered generic and would be modified to account for specific circumstances of a pesticide use in a given risk assessment. Furthermore, the examples provided here do not account for all types of pesticides and/or application methods.

2.2.2.1 Non-systemic, Foliar Spray Applications

For non-systemic pesticides applied via foliar spray, dominant exposure routes of foraging bees include direct deposition of spray droplets onto bees, deposition onto plant surfaces (leaf, flower, pollen, nectar, extra-floral nectaries) followed by contact and/or ingestion, and inhalation of gaseous phase chemical (for highly volatile pesticides; **Figure 4**). Exposure of honey bees via soil residues is not expected to be a major source of exposure to non-systemic pesticides due to limited interaction of honey bees with soil and lack of translocation of pesticides into plant tissue; however, this route is likely important for non-*Apis* ground-nesting bees. Foraging honey bees may also be exposed to non-systemic pesticides via consumption of water from dew droplet formation on leaves, puddles, and other surface water[6]. However, a recent review of exposure routes of honey bees indicated high uncertainty in the importance of water ingestion relative to other oral ingestion sources of non-systemic and systemic pesticides (*e.g.*, nectar and pollen) due to lack of information on water intake rates by bees and multiple factors that affect these rates (EFSA 2012). Dominant exposure routes of hive bees (*e.g.*, nurse, worker, drone bees) include ingestion and processing of pollen and nectar and exposure through production and contact with comb wax. Stored honey is expected to be an important exposure route for over wintering bees. Processed bee bread, brood food, and royal jelly are major routes of exposure for developing larvae and the queen. Although for highly volatile pesticides, hive bees may be exposed via inhalation of gaseous phase chemical, this potential route of exposure is not included in the conceptual model since this would require hives to be in relatively close proximity to the treated site. For example, while soil fumigants typically are among the pesticides with the greatest volatility, their application to fields with bare soil (often under tarped or deep shank injection) suggests that honey bees would not likely be foraging in the treated fields.

Changes in the assessment endpoints (*e.g.*, size and stability of bee colonies, production of hive products, pollinator species richness and abundance) as a result of the aforementioned pesticide exposures may occur through various means, including reduction in number of worker bees available for

[6] For non-systemic pesticides, exposure via water of guttation and honey dew is expected to be minor relative to other routes of exposure.

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foraging or maintaining hive temperature (over wintering), reduction in foraging efficiency via sublethal effects on workers, decreased number or delayed development of brood either from direct exposure to pesticide or indirectly from reduced brood feeding and maintenance by hive bees, and reduced fecundity and survival of queens. Protecting *Apis* populations from pesticide-related effects would also be expected to directly protect other arthropod pollinators with similar exposure and effects profiles. Indirectly, the central role of *Apis* in the propagation of numerous flowering plants would also contribute to species diversity of other non-*Apis* bees. Changes in these assessment endpoints are directly related to impacts on protection goals of maintaining pollination services, production of hive products and contribution to pollinator biodiversity.

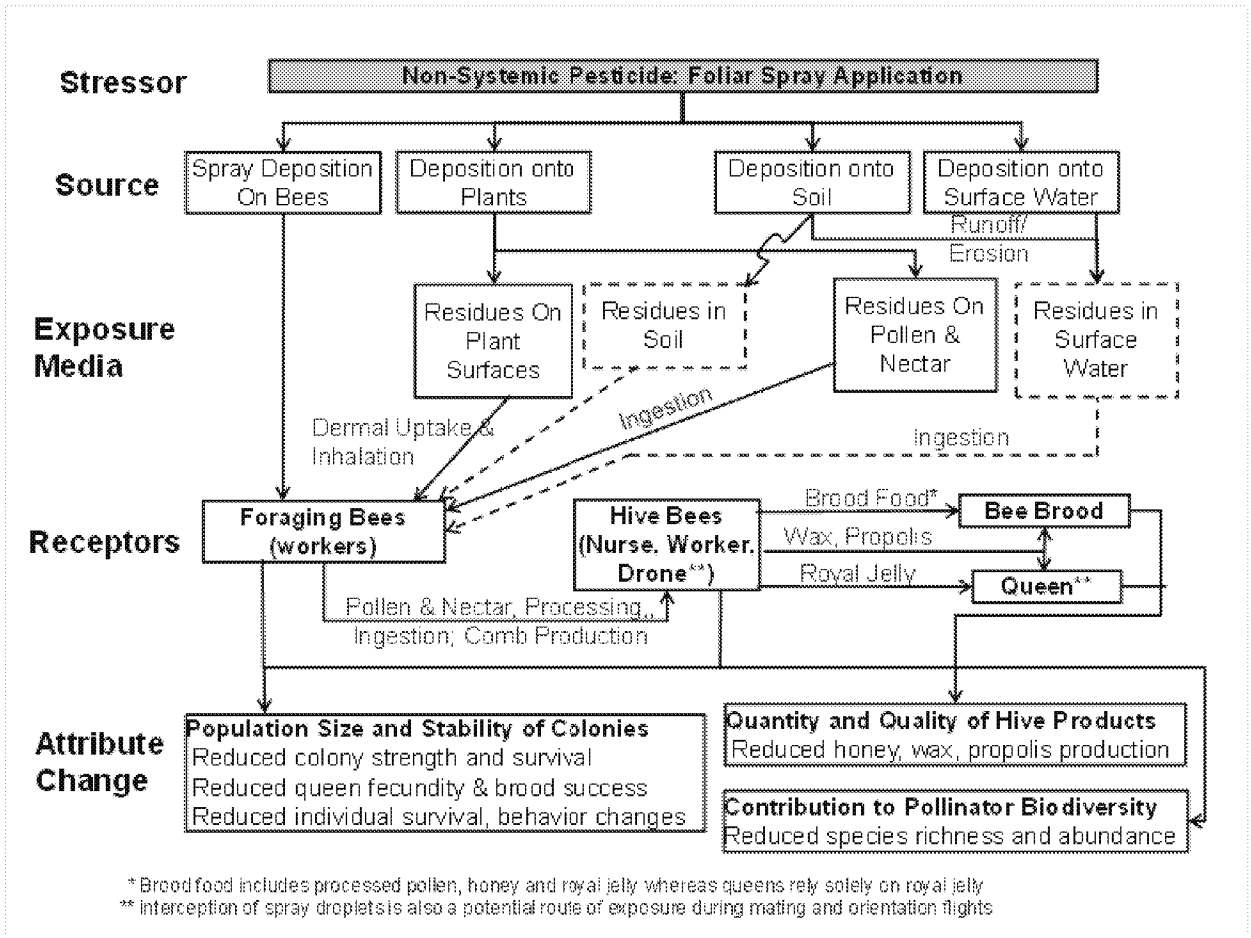

Figure 4. Generic Conceptual Model of Non-Systemic, Foliar-Applied Pesticides for Honey Bee Risk Assessment. Dashed lines represent routes of exposure that are not considered to be major.

2.2.2.2 Systemic, Foliar Spray Applications

Foliar applications of systemic pesticides are likely to result in many of the same routes of exposure to honey bees as described previously for non-systemic, foliar-applied pesticides, with several important

exceptions. First, deposition onto plant surfaces and soil will lead to translocation of the pesticide to other plant tissues, potentially contributing to higher quantities of pesticide residues in pollen and nectar. For persistent systemic pesticides, the exposure window could include longer periods of time (red arrows, **Figure 5**) compared to similar applications of non-systemic pesticides. Second, pesticide residues in plant exudates (guttation fluid, honey dew) also become a potentially relevant route of exposure. As discussed in the previous section, uncertainty and variability in the extent to which honey bees harvest and use guttation fluid (relative to other available sources of water) leads to ambiguity in the importance of this exposure pathway to honey bees. The translation of exposure into changes in assessment endpoints is essentially the same as that described for non-systemic pesticides. However, the timing of exposure to residues in pollen and nectar may persist for significant periods of time after pesticide application, depending on the systemic and persistence characteristics of the pesticide.

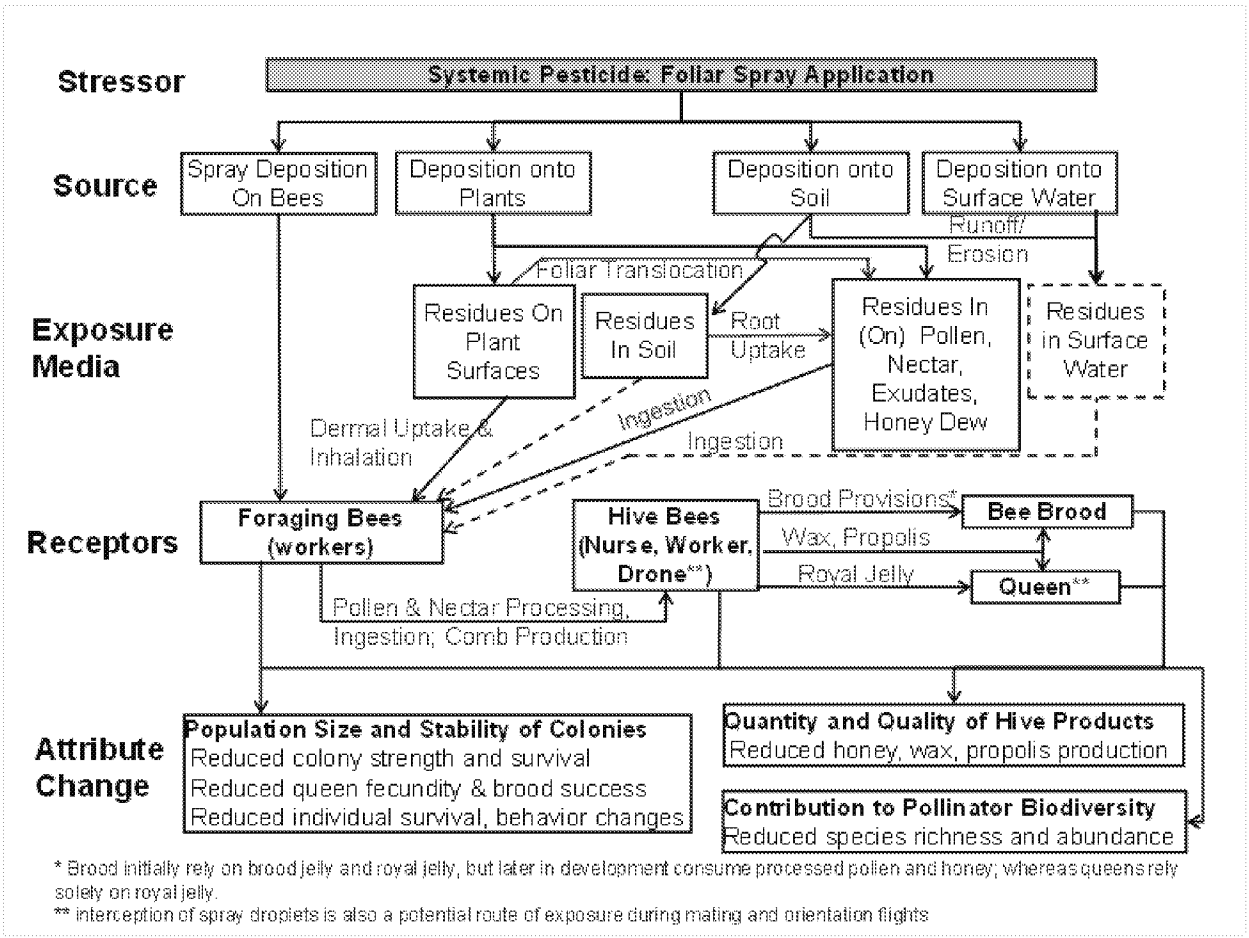

Figure 5. Generic Conceptual Model of Systemic, Foliar-Applied Pesticides for Honey Bee Risk Assessment. Red depicts systemic pathways. Dashed lines represent routes of exposure that are not considered to be major.

2.2.2.3 Systemic, Seed Treatment

Major exposure routes of honey bees to systemic pesticides used as seed treatments include pollen, nectar, exudates (*e.g.*, guttation fluid), and honey dew resulting from translocation from the seed to growing plant tissues **(Figure 6)**. Another important route of exposure includes contact with abraded seed coat dust during planting. The latter pathway has been associated with numerous incidents of honey bee mortality (Pistorius *et al.* 2009, Forster *et al.* 2009) and is the focus of considerable research (*e.g.*, Taparro *et al.* 2012, Krupke *et al.* 2012). The extent to which honey bees are exposed via contact with abraded seed coat dust is determined by many factors including the physico-chemical properties of the seed coating, seed planting equipment, use of seed delivery agents (*e.g.*, talc), environmental conditions (wind speed, humidity), and hive location in relation to sowing. Off-site drift of contaminated seed coat dust also may contribute to residues on plants, soil, and surface water to which bees may be exposed through direct contact and ingestion of surface water, pollen, and nectar. One important attribute of the seed treatment exposure pathway is that exposure to pesticides may occur over a wide time scale (*e.g.*, at seed sowing, during plant growth and flowering, and potentially at plant harvest from exposure to contaminated plant dust). Furthermore, seeds may be coated with multiple fungicides and insecticides which results in simultaneous exposure to pesticide mixtures.

2.2.2.4 Systemic, Soil Application and Tree Application

Systemic pesticides are also applied as soil applications (*e.g.*, soil drench) (**Figure 7**) and less commonly, via trunk drench or injection (**Figure 8**). Exposure of honey bees to pesticides via these applications are expected to result primarily from translocation to plant tissues (pollen, nectar, exudates, and honey dew). For soil applications, there is potential exposure via runoff and subsequent translocation into plants adjacent to the treated field.

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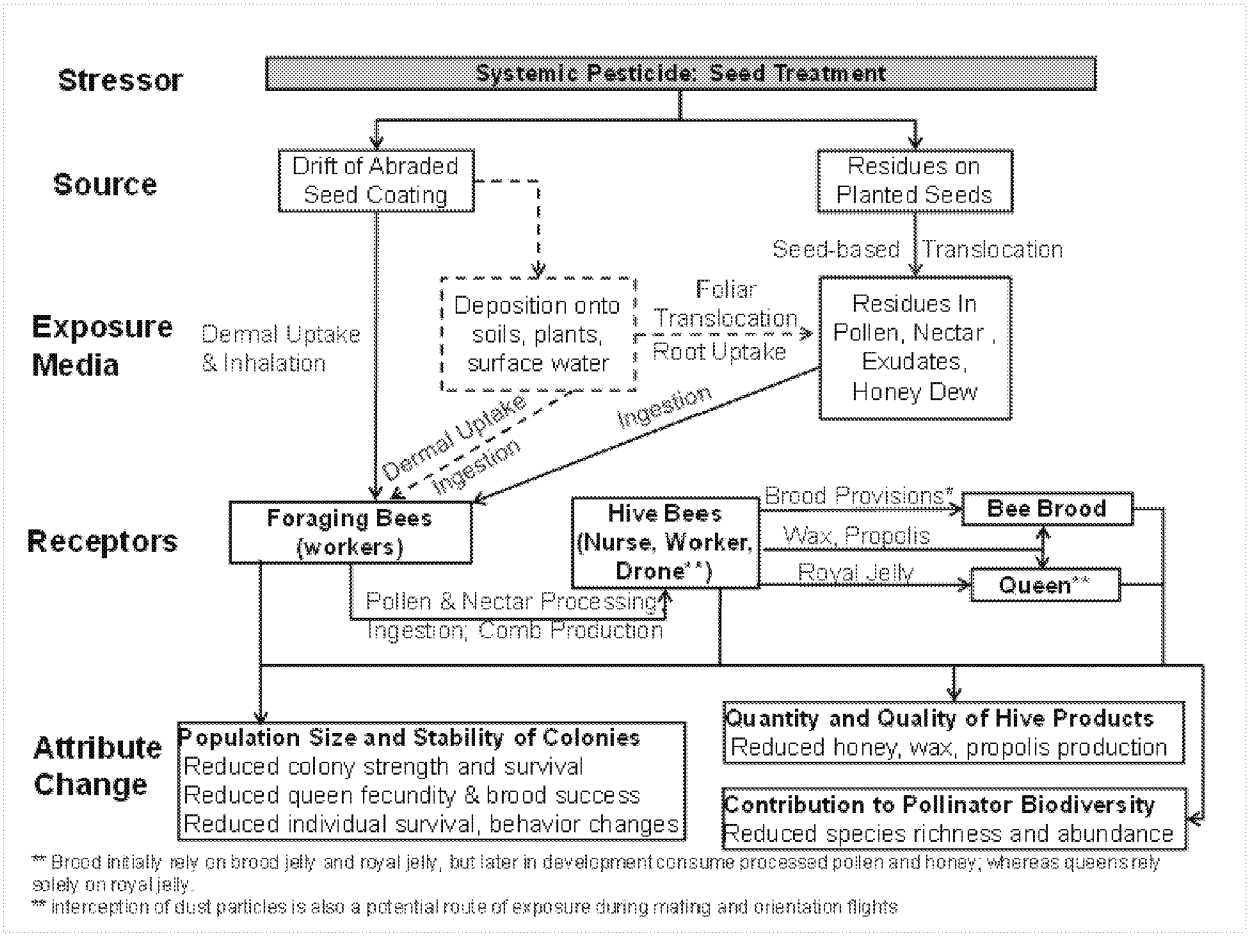

Figure 6. Generic Conceptual Model of Systemic, Foliar-Applied Pesticides for Honey Bee Risk Assessment. Red depicts systemic pathways. Dashed lines represent routes of exposure that are not considered to be major.

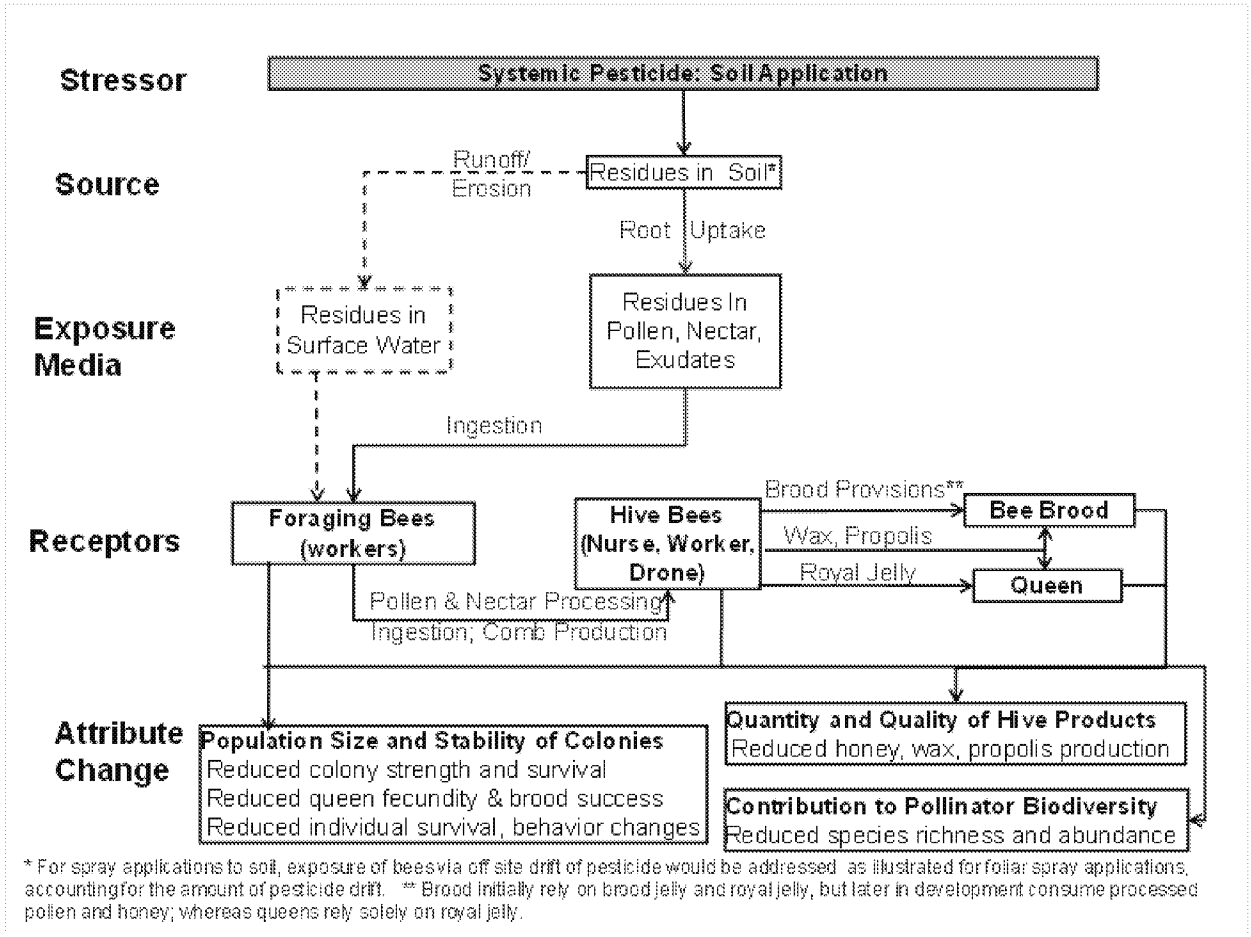

Figure 7. Conceptual Model of Soil-Applied Systemic Pesticides for Honey Bee Risk Assessment. Red depicts systemic pathways. Dashed lines represent routes of exposure that are not considered to be major.

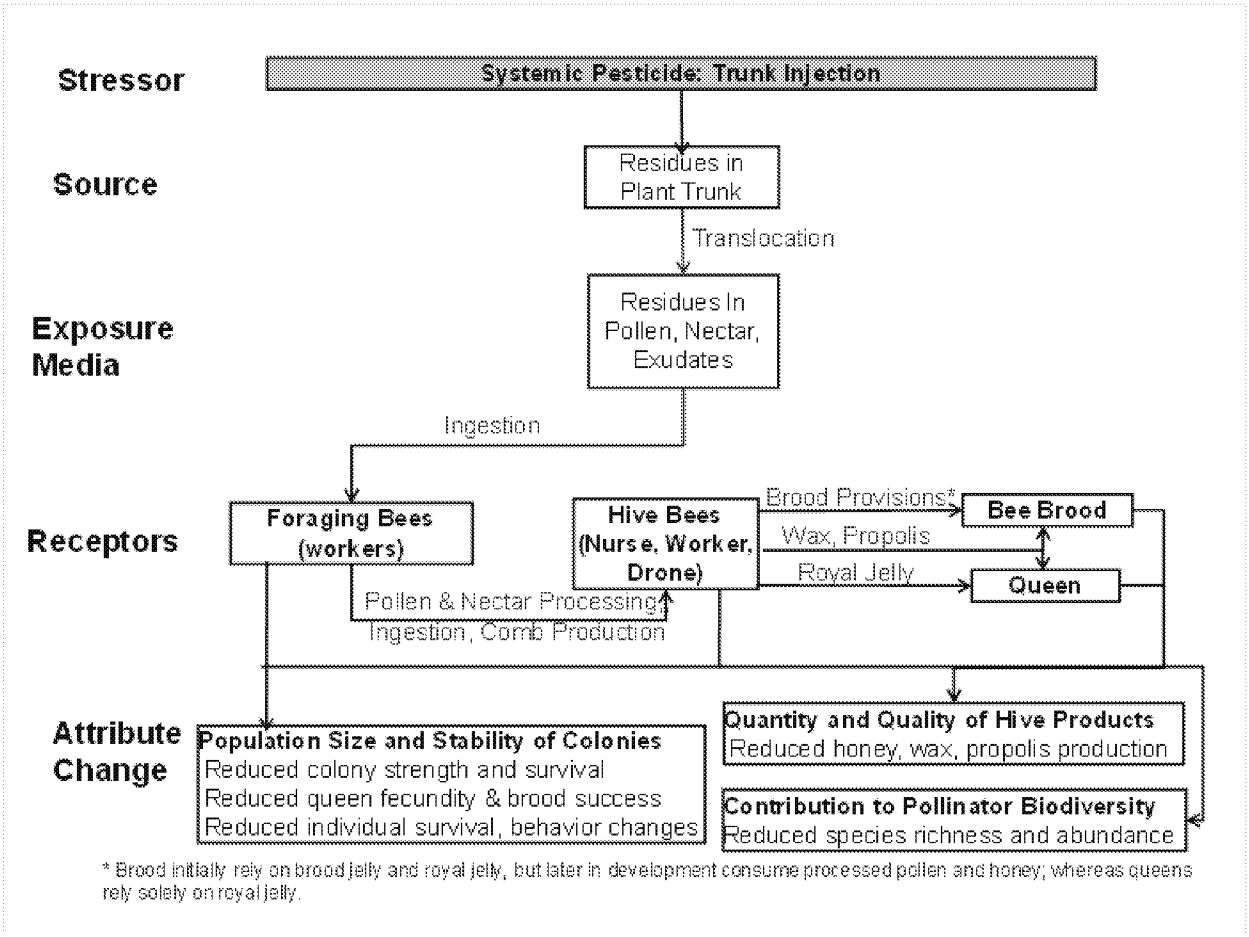

Figure 8. Conceptual Model of Trunk-Injected or Trunk-Drench Systemic Pesticides for Honey bee Risk Assessment. Red depicts systemic pathways.

2.2.3 Analysis Plan

The intent of the problem formulation's analysis plan of a risk assessment is to summarize what has been done during the problem formulation and to focus on those risk hypotheses which are considered most likely to contribute to the actual risk associated with a particular use of a pesticide. The analysis plan provides a rationale for selecting and omitting risk hypotheses in the actual analysis. As with any risk assessment process, the analysis plan also articulates data gaps, the methods used to evaluate existing and anticipated data, and the assumptions that will be made where data may be missing. The analysis plan also identifies the specific measures of exposure (*e.g.*, estimated environmental concentrations; EECs) and effect (*e.g.*, median lethal dose for 50% of the organisms tested; LD_{50}) which will be used to develop risk estimates. As with other taxa, the measures of exposure and effects are not likely to be comprehensive and uncertainties associated with the available data and underlying assumptions must be clearly articulated.

2.2.3.1 Measures of Exposure

Measures of exposure are data which detail a pesticide's existence and movement in the environment and its contact or co-occurrence with the assessment endpoint or its associated measurement endpoint (USEPA 1998). While EPA has routinely generated EECs with increasing levels of refinement for other terrestrial and aquatic taxa, it has not had a similar tiered process for bees. Therefore, in the section to follow, greater detail is devoted to describing measures of exposure to support the screening-level risk assessment process and how these measures can be refined to represent more environmentally realistic estimates based on the needs of risk managers. The proposed measures of exposure make use of existing tools such as the T-REX model as well as approaches that have not previously been used for regulatory purposes in North America for estimating exposure through ingestion of pollen and nectar containing residues of a systemic pesticide applied to soil or seeds.

2.2.3.2 Measures of Effect

Measures of effect are data which describe the effect of a chemical on individuals, species, populations and communities in the ecosystem (USEPA 1998). They are intended to reflect consistent and reliable measurable changes in an attribute on an assessment endpoint or its surrogate in response to a pesticide to which it is exposed. In the past, risk assessors have relied heavily on laboratory-based studies with individual bees to evaluate potential hazards to bees and only had limited opportunity to

evaluate potential effects at the colony level. While the laboratory studies will continue to play an important role in the assessment process, the proposed risk assessment process for bees will articulate additional data which should be considered as well as the triggers that will be used to recommend additional studies. As discussed in the following sections, some of the additional data may already be available since these studies are currently required by Canada and the EU.

Effects data used in the assessment are not confined to registrant-submitted studies but include studies published in the open literature. The open literature studies are identified using EPA's ECOTOXicology (ECOTOX) database, which is maintained by the EPA Office of Research and Development and which employs a literature search engine for locating chemical toxicity data for a range of taxa including bees (USEPA, 2009*a*). EPA has developed guidance (USEPA 2011) for evaluating ecological toxicity data reported in open literature studies and this guidance includes specific information related to studies conducted with bees.

The proposed process for estimating risk to bees focuses on the honey bee, which has historically served as a surrogate for both *Apis* and non-*Apis* bees. Regulatory agencies rely heavily on the use of surrogate species to evaluate potential adverse effects to taxa since well-defined toxicity tests have been developed using particular species. The use of these test species is based on well-defined husbandry conditions which allow the organisms to thrive under laboratory conditions. Additionally, the test species must be readily available and relatively easy to manipulate. All of these conditions influence the extent to which a particular organism can be routinely and reliably used to estimate the toxicity of chemicals without unreasonable confounding effects. The honey bee is readily available, its husbandry needs are very well defined and given that it has been domesticated for many centuries, both the individual bee and the more complex bee colony lend themselves to toxicity testing. The honey bee also serves as a reasonable choice for evaluating the protection goals defined earlier because the species is responsible for a large proportion of crop pollination in North America and produces honey. The use of any species as a surrogate is predicated on the understanding that while the organism may thrive under laboratory and field test conditions, it may not be representative of the most sensitive species or of species with markedly different life histories and pesticide exposure routes. Although the honey bee has historically served as a surrogate for *Apis* as well as non-*Apis* bees, there are limitations in the use of honey bees as surrogates and these limitations and potential means of addressing them are discussed later in this white paper. Also, to the extent that alternative data on the effects of the pesticide on non-

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target arthropod are available, this information can be used to further characterize the extent to which honey bee data may or may not be adequate for assessing effects to non-*Apis* bees.

2.2.3.3 Integrating Exposure and Effects

The proposed screening-level (Tier I) risk assessment process will rely on the point estimate (deterministic) based approach using the ratio of exposure concentration to effects concentration to derive risk quotients (RQs) that serve as a basis for estimating risk to other species of insect pollinators. In cases where RQs exceed the Level of Concern (LOC), risks of the pesticide to insect pollinators cannot be precluded and additional refinements may be necessary. The proposed LOC is set to 0.4 and is based on the historic average dose-response relationship for acute toxicity studies with bees and a 10% mortality level in foragers and worker larvae. When acceptable chronic toxicity test designs are available, the proposed process involves developing chronic RQs using no observed adverse effect concentrations (NOAECs) and evaluating the RQs using an LOC of 1.

Higher tier assessments will be based on a qualitative approach (*i.e.*, RQs not directly calculated) to refine the risk estimates in the lower tier assessments. Given the protection goals and assessment endpoints discussed earlier, risk will be assessed through examining the likelihood of acute mortality and chronic effects on survival, development and reproduction of bees/colonies. Measures of effect will include the acute contact and/or oral LD_{50} from laboratory studies with individual bees using currently available acute toxicity test guidelines, and the most sensitive chronic NOAEC and lowest observed adverse effects concentration (LOAEC) for survival, growth or reproduction from chronic toxicity studies when suitable study designs are developed. Measurement endpoints for screening-level assessments will be based on individual bees (adults and/or larvae), while higher tier assessments are expected to provide colony-level NOAEC/LOAEC values. As with other taxa, data from acceptable registrant-submitted and open literature laboratory and field-based studies and the resulting RQ values will be considered in the context of any ecological incident data (*e.g.*, bee kills) which may be available in the EPA Ecological Incident Information System (EIIS) and other incident databases.

One tool that is being considered for the risk assessment method for bees is a colony-level simulation model. This tool may be useful for integrating the wealth of biological information on honey bees and their colonies with the lethal and sublethal effects data for pesticides. A colony-level model would be useful in evaluating the effects of a chemical on the stated protection goals by evaluating impacts of a

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chemical on colony survival, pollination services and honey production. Although no specific colony-level models are proposed for use at this time, they could potentially be developed to integrate exposure and effects data in the evaluation of risks of a pesticide.

After the Problem Formulation has articulated the protection goals, conceptual model/risk hypothesis and associated assessment/measurement endpoints, the risk assessment proceeds to the Analysis step where available exposure and effects data are assessed and characterized. In the following sections, the two components on Analysis (*i.e.*, exposure characterization and effects characterization) are discussed. Although EPA has historically evaluated potential hazards to bees using existing registrant-submitted guideline toxicity studies as well as open literature studies and incident data, estimates of potential exposure to bees have not been routinely evaluated by EPA. Therefore, a more detailed discussion of methods to estimate exposure is provided in the exposure characterization section; whereas, the effects characterization section focuses on study design elements to improve/expand existing guideline studies.

3 Characterization of Exposure

This section describes two Tiers that may be used to characterize potential pesticide exposures to honey bees. The first Tier is intended to efficiently identify those pesticides that do not pose a risk to pollinators. In regards to exposure, the Tier I screen is based on upper-bound estimates of exposure to bees through diet or direct spray. In this approach, surrogate data (*i.e.,* based on other chemicals and plant matrices besides pollen and nectar) are used to represent potential exposures for assessed pesticides. The proposed Tier I exposure method is efficient in that it is easy to use, requires few input parameters, and takes little time to complete.

For chemicals that fail the Tier I screen, prior to moving on to the Tier II assessment, it may be possible to utilize pesticide-specific data from registrant-submitted studies describing pesticide residues in plants (*e.g.,* magnitude of residues, OSCPP 860.1500; USEPA 1996*a*).These studies could potentially provide enough information to allow for adequate characterization of the risks of the pesticide without needing to move on to Tier II, which would preclude the need to require additional pesticide-specific field studies. If the existing registrant-submitted studies do not provide sufficient information to characterize the risks of the pesticide to honey bees, the data could potentially be used to inform the study designs of the Tier II studies. These refinements may involve gathering and evaluating large amounts of chemical

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specific data. This step is not efficient; however, it may provide valuable information that may be used to characterize the risk of a chemical to honey bees.

Tier II exposure assessments rely upon field measurements of the assessed pesticide in pollen and nectar of crops that are relevant to the use of the pesticide and the exposure of bees (*e.g.*, crops that are attractive to bees). In cases where sufficient pesticide residue data for pollen and nectar are submitted prior to risk assessment, it may not be necessary for the risk assessor to use the Tier I screen since pesticide-specific residues in pollen and nectar could be used directly to understand potential exposures to honey bees.

This section describes the proposed Tier I exposure assessment method for honey bees, which includes a discussion of how registrant-submitted field studies that are submitted to meet identified data needs not specifically related to honey bees (*i.e.*, for determining potential pesticide exposures to humans), could potentially be used to characterize risks that could not be precluded by the Tier I screen. The final component of this section is a discussion of the Tier II exposure options, with a focus on considerations related to Tier II study designs.

3.1 Tier I Exposure Assessment for Honey Bees

The purpose of this section is to describe a screening level approach that is proposed as a Tier I method to assess exposures of honey bees to pesticides. The Tier I exposure method is intended to account for the major routes of pesticide exposure that are relevant to bees (*i.e.*, through diet and contact). As indicated by the conceptual models, the exposure routes for bees are expected to differ based on application type. For instance, honey bees foraging in a field treated with a pesticide through foliar spray could potentially be exposed to the pesticide through direct spray as well through consuming contaminated food. For honey bees foraging in fields treated with a pesticide through direct application to soil (*e.g.*, drip irrigation), or through seed treatments, direct spray onto bees is not expected. For these application methods, pesticide exposure through consumption of residues in nectar and pollen are expected to be the dominant routes. The conceptual models discussed previously in this white paper acknowledge that foraging honey bees may also be exposed to pesticides via contact with dust from seed treatments or via consumption of water from surface water, puddles, dew droplet formation on

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leaves and guttation fluid. As explained below, the proposed Tier I exposure methods do not include quantification of exposures via these routes.

As discussed in the effects characterization, acute oral toxicity data are necessary for adult and larvae in order to characterize the risks of a pesticide. Because these toxicity data are expressed on a dose basis (*i.e.*, μg a.i./bee), it is necessary to convert estimated concentrations of pesticides in food (expressed as mg a.i./kg) into doses. Honey bees fulfill their nutritional requirements through consumption of nectar, honey, pollen and bee bread. In addition to requiring pollen and nectar or honey, bees also require royal jelly and brood food to fulfill their nutritional requirements. In the proposed approach, pesticide doses received by bees can be calculated using nectar and pollen consumption rates for larval and adult worker bees. For larvae, the proposed total food consumption rate is 120 mg/day, which is based on the total daily consumption of pollen and nectar (based on honey consumption) by larvae during day 5 of the uncapped larval life stage. For adult worker bees, the proposed food consumption rate is 292 mg/day, based on nectar consumption rates of nectar foraging bees, which are expected to receive the highest dietary exposures among different types of worker bees. In addition, it is likely that these food consumption rates are protective of drones and queens. A detailed discussion related to the derivation of the proposed food consumption rates for larvae and adult workers is provided in **Appendix 1**, along with a comparison of the proposed rates to other types of worker bees and castes.

In the proposed approach for representing food consumption rates of larvae and adults, it is assumed that exposures through consumption of nectar and pollen are conservative representations of potential exposures through consumption of honey and bee bread, respectively. This approach is likely to be conservative because it assumes that pesticides do not degrade while honey and bee bread are stored in the hive. For bees that consume honey, it is assumed that the estimated pesticide exposures can be related back to the original concentration in nectar by accounting for the amount of sugar consumed by bees. It is also assumed that pollen and nectar consumption rates and resulting exposures are protective of exposures of bees to pesticides through consumption of royal jelly and brood food. This is supported by work by Davis and Shuel 1988 and Kamel *et al.* (unpublished) demonstrating that pesticide concentrations in food consumed by nurse bees are 2-4 orders of magnitude higher than concentrations measured in royal jelly.

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Methods for estimating dietary exposures to bees differ in the nature of the estimated concentrations in pollen and nectar potentially consumed by bees. For foliar spray applications, the proposed approach involves the use of the tall grass residue value from the T-REX model (v. 1.5) as a surrogate for pesticide concentrations in nectar and pollen. Also discussed below is the potential for deriving a pollen-specific upper-bound concentration to represent concentrations of pesticides on pollen and nectar of flowers directly sprayed with pesticides. For soil treatments, the proposed method is based on a modification to a plant-soil uptake model developed by Briggs *et al.* 1982 and 1983, which is designed to estimate pesticide concentrations in plant shoots; the concentrations in plant shoots are proposed as a surrogate for concentrations in pollen and nectar (following systemic transport). For seed treatments, the proposed Tier I exposure method is based on the International Commission for Plant-Bee Relationships' (ICP-BR) 1 mg a.i./kg concentration to represent an upper-bound concentration in nectar and pollen. For tree injections and trunk drenches, the proposed method is a simplistic approach that considers the mass of the pesticide applied to a tree and the mass of the leaves of the tree.

The proposed Tier I methods for foliar spray applications, soil applications, seed treatments and tree injections/drenches are described in detail below, along with comparisons of estimated values to empirical data and a discussion of the strengths and limitations of the methods. The model evaluations described below discuss available empirical measurements of pesticides in pollen, nectar and on bees. To compare the estimated exposures to the empirical measurements in nectar and pollen and on bees, all concentrations were normalized to 1 lb a.i./A. In addition, the Tier I exposure estimates are converted to a dose basis (μg a.i./bee) to facilitate the comparison of the exposure value to toxicity data, the latter of which are expressed on a dose basis. For reference purposes, summaries of the studies from registrant-submissions and the scientific literature used to evaluate Tier I model predictions are provided in **Appendices 3 and 4**, respectively.

The Tier I method is intended to generate "reasonably conservative" estimates of pesticide exposure to honey bees, meaning that the estimates of exposure should generally be within one or two orders of magnitude higher than the "true" environmental exposure. Models were evaluated by comparing estimated exposures to available empirical data for nectar, pollen and bees to determine whether they could be considered reasonably conservative. The amounts of data that could be used to evaluate the

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different methods varied, which lead to differences in the evaluation of the conservativeness of different methods. For example, for foliar spray applications, the empirical data available to evaluate the proposed methods for estimating exposures through consumption of pollen and nectar (N>15) were much larger than the data for estimating contact exposures (N = 3). In cases where only a few data are available to evaluate a method, it is preferred that the estimated exposure value has a larger margin of safety than the methods that can be evaluated using larger empirical data sets. For the methods with smaller evaluation data sets, there is less confidence in the conservativeness of the method because it is not certain that the small data set captures upper-bound exposures in the environment. For methods that have more empirical evaluation data, the margin of safety between the estimated value and the largest empirical value could be lower as long as the estimated value was conservative for the overall data set because it is more likely that the upper-bound of environmental exposures is represented.

As discussed in the analysis plan of this white paper, estimated exposure concentrations are integrated with available toxicity data in order to characterize risks of a pesticide to honey bees. In doing so, Tier I estimated exposures and toxicity endpoints are compared based on the same exposure routes. For instance, estimated exposures through direct spray onto foraging bees are combined with toxicity endpoints from contact toxicity test, while estimated dietary exposures[7] are matched with oral toxicity data. The effects characterization below discusses both acute and chronic toxicity tests. At the Tier I level, both acute and chronic exposure estimates are represented by the highest single day exposure value. Although a time weighted estimated exposure value may be more representative of the exposure used in a chronic toxicity test, exposure occurring over a single day could potentially be sufficient to elicit effects. Therefore, in the Tier I approach, chronic exposure is conservatively represented by the highest single day estimated exposure.

[7] Note that the term "dietary exposure" is used throughout the exposure characterization section to describe potential exposures of bees through consumption of pollen and nectar. This term is often described in other publications as "oral exposure"; however the term "dietary" is used to distinguish between pesticide exposures through consumption of food and of drinking water.

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3.1.1 Quantifying Pesticide Exposures from Foliar Applications

3.1.1.1 Estimating exposure through consumption of nectar and pollen

In an ideal situation, the Tier I exposure estimates for honey bees would be based on residue values measured directly in nectar and pollen of flowers sprayed with pesticides. This cannot be achieved at this time because there is an insufficient amount of data that may be used to adequately describe the distribution of pesticide residues that occur in pollen and nectar relative to pesticide application rate. As an alternative, the proposed method relies on upper-bound pesticide residue values from EPA's T-REX model (version 1.5) (USEPA 2012*e*). T-REX (version 1.5) is a Tier I model currently used by EPA to determine potential risks of pesticide exposures to non-target animals located in terrestrial environments, including the treated field or areas receiving spray drift. This model is used for pesticides that are applied via aerial or ground spray applications. The T-REX model estimates pesticide exposures to mammals, birds, terrestrial-phase amphibians and reptiles that are consuming dietary items containing pesticide residues. T-REX is simple and efficient, requiring only a few input parameters related to the application of the chemical (*i.e.*, application rate, number of applications and interval) and its fate (*i.e.*, foliar dissipation half-life). This model generates estimates of exposure that can be expressed as mg a.i./kg-food item and can be related to application rate by multiplying a default residue value that is normalized to 1 lb a.i./A (1.12 kg a.i./ha) by the application rate. Dietary items included in the model are grass (short and tall), broadleaf plants, fruit, pods, seeds and arthropods. The normalized upper-bound and mean residue values for these food items are provided in **Table 2**.

Table 2. Application rate normalized upper-bound and mean initial residue values incorporated into T-REX (v. 1.5.1) for various food items.

Terrestrial animal food item	Initial residue (mg a.i./kg per 1 lb a.i./A)	
	Upper-bound	Mean
Short grass	240	85
Broadleaf plants	135	45
Tall grass	110	36
Arthropods	94	65
Fruit, pods and seeds	15	7

For the plant dietary items, this model incorporates the Kenega nomograph, as modified by Fletcher *et al.* (1994), which is based on a large set of field residue data. The upper-limit values from the nomograph represent the upper bound of residue values from actual field measurements (Hoerger and Kenega 1972). The Fletcher *et al.* 1994 modifications to the Kenega nomograph are based on measured

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field residues from 249 published research papers, including information on 118 species of plants, 121 pesticides, and 17 chemical classes.

The T-REX model is of particular interest for the Tier I exposure approach for bees because it is based on empirical data from field and laboratory studies, represents a conservative approach to deriving pesticide residue estimates on plants and arthropods, is part of EPA's standard Tier I ecological risk assessment method, and can be modified to account for the application rate and number of applications of a pesticide. Since the T-REX model does not specifically include residue values for pollen and nectar, it is necessary to select an appropriate plant residue value to act as a surrogate for these matrices. The sections below compare the residues on different T-REX plant categories to empirically-based pesticide concentrations in pollen and nectar of flowers that were directly sprayed. As noted in the effects section below, Tier I level acute oral toxicity data are potentially submitted for two life stages of worker bees, *i.e.*, adults and larvae. Therefore, for the Tier I exposure method, two estimated dietary doses are generated to represent the two life stages. Since both of these life stages consume nectar and pollen to varying extents, it is proposed that dietary exposures be assessed as a combination of the two food sources.

3.1.1.1.1 Concentrations in Nectar after Foliar Applications

Empirical data for pesticide concentrations in nectar from flowers that were directly sprayed with pesticides were compiled in order to evaluate the potential utility of the T-REX upper-bound residues in short grass, tall grass, broadleaf plants and fruit, seeds and pods as potential screens for assessing dietary exposures to honey bees. These data are listed in **Table 3** and summaries of the studies used to generate the data are provided for reference purposes in **Appendices 3 and 4**. Empirical measurements of eight different pesticides in nectar are available from seven studies (Choudhary and Sharma 2008, Wallner 2009, and 5 unpublished registrant-submitted studies involving chemicals whose names are not provided here because the chemicals are not currently registered). The highest empirical measurement from these studies was 13.6 mg/kg-nectar (normalized to 1 lb a.i./A) (**Table 3**); however, it should be noted that this value corresponds to a mean measurement of pesticides in nectar, not to a maximum value. Therefore, it is more appropriate to compare this value to the mean residues used by the T-REX model, rather than the upper bound. Despite this limitation, the mean value is useful to describe potential residues of pesticides in nectar.

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Table 3. Comparison of T-REX upper-bound values for plants and measured concentrations (maximum and mean values) of pesticides in nectar.

Citation	Normalized Concentration (mg/kg per 1 lb a.i./A)		Chemical	Crop
	Maximum	Mean		
T-REX (short grass)	240*	85	All	All
T-REX (Broadleaf plants)	135*	45	All	All
T-REX (Tall grass)	110*	36	All	All
Choudhary and Sharma (2008)	NA	13.6	*Lambda* cyhalothrin+	Mustard
Choudhary and Sharma (2008)	NA	12.5	*Lambda* cyhalothrin+	Mustard
Choudhary and Sharma (2008)	NA	7.22	Spiromesifen+	Mustard
Choudhary and Sharma (2008)	NA	7.03	Spiromesifen+	Mustard
T-REX (Fruit, pods and seeds)	15*	7	All	All
Choudhary and Sharma (2008)	NA	3.90	Endosulfan+	Mustard
Choudhary and Sharma (2008)	NA	3.45	Endosulfan+	Mustard
Wallner (2009)	NA	3.2	Boscalid	Oilseed rape
Wallner (2009)	NA	3.2	Prothioconazol	Oilseed rape
Study 1-2+	2.2	2.1	Unnamed chem #1	*Phacelia*
Study 1-2+	2.1	1.5	Unnamed chem #1	*Phacelia*
Beedle and Harbin (2011)	1.1	NA	Imidacloprid**	Cotton
Study 1-1	0.83	NA	Unnamed chem #1	Cotton
Study 2-1	0.61	NA	Unnamed chem #2	*Phacelia*
Study 1-1+	0.41	NA	Unnamed chem #1	Cotton
Study 1-1+	0.41	NA	Unnamed chem #1	Cotton
Study 2-2	0.17	NA	Unnamed chem #2	Melon

*Value represents upper bound, not maximum.
**Includes parents and degradates.
+This study included two different trials. The results for each trial are presented separately.
NA = not available

When comparing the maximum values from the available empirical data to the T-REX upper-bound residue values for plants, the T-REX residue estimates exceed the maximum empirical values. When considering cases where only means were provided in study reports, the T-REX mean values exceed the empirical means for short grass, broadleaf plants and tall grass. Since several mean values from empirical studies exceed the mean residue value for seeds, fruit and pods, this T-REX residue value may not be protective of residue values in nectar. The next highest residue value from T-REX, which is 110 mg/kg normalized to 1 lb a.i./A (1.12 kg a.i./ha), corresponds to an upper bound of pesticides measured

on tall grass. The mean value for short grass exceeds all empirical mean data, and is within a factor of 3 of the highest mean value. This indicates that this value would serve as a screen that is a reasonably conservative estimate of the pesticide dose received by bees consuming nectar. Therefore, the T-REX screening value of 110 mg/kg (per 1 lb a.i./A or 1.12 kg a.i./ha) is proposed as a surrogate for nectar.

3.1.1.1.2 Concentrations in Pollen after Foliar Applications

Empirical data for pesticide concentrations in pollen from flowers that were directly sprayed with pesticides were compiled in order to evaluate the potential utility of the T-REX upper-bound residues in short grass, tall grass, broadleaf plants and fruit, seeds and pods as potential screens for assessing dietary exposures to honey bees. The T-REX surrogates are evaluated using empirical measurements of 11 different pesticides in pollen from studies available in the open literature (Choudhary and Sharma 2008, Fries and Wibran 1986, Hanny and Harvey 1982, Škerl *et al.* 2009 and Wallner 2009) or from unpublished studies submitted by pesticide registrants. These data are listed in **Table 4** and study summaries are provided in **Appendices 3** and **4**.

Table 4. Comparison of T-REX upper-bound values for plants and measured concentrations (maximum and mean values) of pesticides in pollen measured within 24 hours of application.

Citation	Normalized Concentration (mg/kg per 1 lb a.i./A)		Chemical	Crop
	Maximum	Mean		
T-REX (short grass)	240*	85	All	All
Wallner 2009	NA	58	Boscalid	Oilseed rape
T-REX (Broadleaf plants)	135*	45	All	All
T-REX (Tall grass)	110*	36	All	All
Fries and Wibran 1987	50	NA	PP321	Oilseed rape
Fries and Wibran 1987	49	NA	Cypermethrin	Oilseed rape
Study 2-1[+]	38	NA	Unnamed chem #2	*Phacelia*
Study 1-1[+]	31	NA	Unnamed chem #1	Cotton
Study 1-1[+]	17	NA	Unnamed chem #1	Cotton
Choudhary and Sharma 2008 [+]	NA	25.0	*Lambda* cyhalothrin[+]	Mustard
Choudhary and Sharma 2008[+]	NA	24.1	*Lambda* cyhalothrin[+]	Mustard
Study 2-1[+]	19	NA	Unnamed chem #2	*Phacelia*
Study 1-2[+]	19	NA	Unnamed chem #1	*Phacelia*
T-REX (Fruit, pods and seeds)	15*	7	All	All
Study 1-2[+]	14	NA	Unnamed chem #1	*Phacelia*
Study 1-1[+]	12	NA	Unnamed chem #1	Cotton
Choudhary and Sharma 2008 [+]	NA	10.5	Spiromesifen[+]	Mustard

Study 1-1+	10	NA	Unnamed chem #1	Cotton
Choudhary and Sharma 2008 +	NA	9.09	Spiromesifen+	Mustard
Choudhary and Sharma 2008 +	NA	4.75	Endosulfan	Mustard
Choudhary and Sharma 2008 +	NA	4.54	Endosulfan	Mustard
Hanny and Harvey 1982	NA	3.1	Carbaryl**	Corn
Hanny and Harvey 1982	NA	1.6	Carbaryl**	Corn
Škerl *et al.* 2009	1.5	NA	diazinon	Apple
Study 2-2	1.21	NA	Unnamed chem #2	Melon
Study 2-3	1.29	NA	Unnamed chem #2	Tomato
Škerl *et al.* 2009	1	NA	Thiacloprid	Apple

*Value represents upper bound, not maximum.
**Two different experiments were conducted using different formulations. The results of the two formulations are presented separately.
+This study included two different trials (for each pesticide). The results for each trial are presented separately.
NA = not available

When comparing the empirical data to the T-REX upper-bound residue values for plants (**Table 4**), the T-REX residues values for short grass, tall grass and broadleaf plants exceed the maximum empirical data for pollen. The highest maximum value is within a factor of 2 of the tall grass upper-bound value. The upper-bound residue for seeds, fruit and pods (15 mg/kg normalized to 1 lb a.i./A or 1.12 kg a.i./ha) is lower than several values from empirical data set for pollen, suggesting that this value is not protective for pollen. When considering the mean empirical data, there is one mean value that exceeds the mean values for tall grass and broadleaf plants; however it is still within a factor of 2 of both of these values. Given that all but one of the mean or maximum empirical data are below the T-REX tall grass mean or upper-bound values (respectively) and that the empirical dataset is composed of a large number of data points (*i.e.*, N = 23) for pollen, it appears that the T-REX upper-bound residue value for tall grass represents a conservative screening estimate of the pesticide dose received by bees consuming pollen. Therefore, the T-REX screening value of 110 mg/kg (per 1 lb a.i./A) will be proposed as a surrogate for pollen.

3.1.1.2 Assumptions and Uncertainties associated with dietary exposure methods for foliar applications

As indicated previously, the Tier I dietary exposure estimates would ideally be based on residue values measured directly in nectar and pollen of flowers sprayed with pesticides. Due to the limited availability of empirical measures of pesticides in nectar and pollen (**Table 3** and **Table 4**, respectively), there is limited confidence in the upper-bound estimates that would be generated using these data. In addition, the available data are not necessarily diverse enough to represent the major variables that are expected

to lead to differences in pesticide concentrations among treatment sites. For example, there may be crop specific factors, such as flower structure or orientation that may impact the magnitude of pesticide concentrations in pollen and nectar of different types of crops. There are an insufficient number studies available for each type of crop that may be used to evaluate the potential influences of these factors. In addition, the available data represent a mixture of maximum and maximum values (where the means are often based on a low number of replicates) making it difficult to combine these data into a unified data set that can be used to reliably estimate 95th percentile concentrations in nectar and pollen. If 95th percentile values were calculated using the maximum values available for nectar and pollen, these values would be 2.2 (N=8) and 49 mg/kg per 1 lb a.i./A (N = 14), respectively (**Table 5**). When compared to the T-REX upper-bound residue value for tall grass (*i.e.,* 110 mg a.i./kg per 1 lb a.i./A), the 95th percentile value for pollen is only a factor of 2 less. Given the limited amount of data available for pollen, it is likely that if the amount of data is increased, the higher concentrations in the data set may be closer to the tall grass upper-bound value. Although the value for nectar appears to be an order of magnitude lower compared to pollen, in some studies where pollen and nectar were collected from the same flowers (*e.g.,* Choudhary and Sharma 2008), the concentrations in the two matrices were almost identical, suggesting that pesticide concentrations in pollen and nectar may be equivalent in some cases.

Table 5. Summary statistics describing concentration data for nectar, pollen and tall grass.

Parameter	Concentration in nectar (mg/kg per 1 lb a.i./A)		Concentration in pollen (mg/kg per 1 lb a.i./A)		Concentration in tall grass (mg/kg per 1 lb a.i./A)*
	Maximum	Mean	Maximum	Mean	Maximum
Maximum	2.2	14	50	58	197
Average	1.0	5.8	19	16	36.0
Standard deviation	0.8	4.3	17	18	40.6
N	8	10	14	9	46
Upper 95th percentile	2.2	13	49	45	110**
Number of plant species	5		8		6
Number of chemicals	8		11		20

*From Fletcher et al. 1994.
**This value represents an approximation of the 95th percentile.

There is uncertainty regarding the extent to which the residues incorporated into T-REX for plants, specifically tall grass, represent biologically relevant surrogates for nectar and pollen. Perhaps a more biologically relevant surrogate for pollen and nectar would be whole flowers. There are some data to suggest that pesticide residues on leaves are similar to those on whole flowers when applied directly to the foliage and flowers via spray (**Table 6**), which supports the use of tall grass as a surrogate for pollen,

despite questions as to the biological relevance of leaves. In the future, as additional pesticide concentration data for nectar and pollen are available, it will be possible to base upper-bound values for nectar and pollen directly on empirical data, thus eliminating the need for the tall grass foliar residue surrogate.

Table 6. Mean pesticide residues measured on leaves and flowers directly sprayed with pesticides. Values based on samples collected on the day of the application.

Citation	Mean (SD) Normalized Concentration (mg/kg per 1 lb a.i./A)		Chemical	Crop
	Leaves	Flowers		
Menkene *et al.,* 1989	46.4 ± 15.5	49.3 ± 17.9	methamidophos	Sugar beets
Perrit *et al.,* 1990	132 ± 80	32 ± 18	methamidophos	Cotton
Delabie *et al.* 1985	44	33	cypermethrin	Oilseed rape

It should be noted that the T-REX model is designed to derive EECs following foliar spray applications of pesticides. Since the data set used to derive the T-REX residue values (Fletcher *et al.* 1994) is based on various spray application methods (*e.g.,* ground boom, air blast, aerial spray), it is assumed that these residues are applicable to any foliar spray application.

Although the data set used to derive the initial residue values of T-REX likely contained systemic pesticides, the residue values are based on measurements that were taken within 24 hours of the application. This leaves little time to allow for transport throughout the plant. Therefore this approach is not applicable to derive EECs representing systemic transport of pesticides after soil or seed treatments.

In using the T-REX residue value, it is assumed that direct spray onto foliage represents a conservative (upper bound) estimate of concentrations in pollen and nectar for both systemic and non-systemic pesticides. Although movement of systemic transport of pesticides to pollen and nectar are expected to occur, concentrations in nectar and pollen that occur due to systemic transport are not expected to exceed the concentrations from direct spray. This is because systemic transport takes time and not all of the mass of the pesticide that is applied to the plant will move to pollen and nectar due to dissipation of the pesticide and transport to other portions of the plant. This assumption is supported by data available for unnamed chemical #1 (study 1-1) and dinotefuran and thiamethoxam (Dively and Kamel 2012). In Study 1-1, cotton blossoms that were directly sprayed with the chemical had higher concentrations in pollen collected from plants (by 2 orders of magnitude) compared to concentrations in

pollen of blossoms that opened after the application, which represents systemically-transported chemical. Concentrations of the pesticide in nectar collected by foraging bees were similar (*i.e.*, within 1 order of magnitude) when comparing flowers that were directly sprayed and flowers that opened after the application, with one exception. In this one case, the pesticide was measured in nectar (from flowers that were not directly sprayed) at a level that was one order of magnitude above the nectar concentration in flowers that were directly sprayed for reasons that are not fully understood. Since the tall grass residue value appears to be higher than available residue data for nectar, the tall grass residue value is expected to still be conservative in cases where systemic transport to nectar may occur. Dively and Kamel 2012 applied dinotefuran and thiamethoxam to pumpkin leaves via foliar spray and measured their residues in pollen and nectar of unsprayed flowers several weeks after the applications. In nectar, maximum residues were 0.015 - 0.080 mg a.i./kg per 1 lb a.i./A. In pollen, maximum residues were 0.082 - 0.95 mg a.i./kg per 1 lb a.i./A. These values are several orders of magnitude below the tall grass upper-bound residue value (*i.e.,* 110 mg a.i./kg per 1 lb a.i./A), which suggests that this residue value is protective for foliar sprays, including cases where pesticides may be systemically transported to pollen and nectar.

3.1.2 Estimating exposure through contact for foliar applications

This section discusses two methods that were considered for the Tier I method to estimate exposure to bees through contact. The proposed upper-bound residue value for estimating exposures to honey bees is based on the maximum residue value reported by Koch and Weisser 1997 (*i.e.,* 2.7 μg a.i./bee per 1 lb a.i./A). When developing the proposed Tier I method for quantifying contact exposure, the upper-bound arthropod residue value from T-REX (*i.e.,* 12 μg a.i./bee per 1 lb a.i./A) was also considered. Both methods are useful in deriving upper bound concentrations of chemicals on insect pollinators, and are in general agreement with each other (they differ by a factor of 5) and with the limited empirical data from other studies reporting pesticide concentrations on bees. Although the T-REX arthropod residue value is relevant to quantifying upper-bound residue values on insects, the utility of this residue value to represent exposures to honey bees is uncertain because the underlying data set does not include measured residues on honey bees. Since the focus of the Tier I risk assessment method is on honey bees (which represent surrogates for non-*Apis* bees), the proposed upper bound residue value for estimating exposures to honey bees is based on the maximum residue value reported by Koch and Weisser 1997 because this value is based on measured concentrations of pesticides on honey bees. In cases where it is

necessary to assess exposures to non-*Apis* bees, the T-REX residue value may be considered more appropriate.

3.1.2.1 Proposed method: Koch and Weisser (1997)

Koch and Weisser 1997 investigated potential contact based exposures of bees to pesticides through applications of a fluorescent tracer (sodium-fluorescein) to flowering apple orchards and *Phacelia tanacetifolia* (commonly referred to as *Phacelia*) fields located in Germany. The mass of tracer present on bees was measured after foraging in flowering apple orchard (n=9) or *Phacelia* fields (n=5) for various time intervals after spray application at 0.018-0.020 lb a.i./A (0.020-0.022 kg a.i./ha). Bees were foraging during spray application, so they could have been directly sprayed with the tracer. For the apple orchards, the mean-measured amount of tracer per bee ranged 1.62-20.84 ng a.i./bee for the nine trials, and an overall average of 6.33 ng a.i./bee. The range of averages is equivalent to 0.079-1.02 μg a.i./bee when normalized to 1 lb a.i./A and the normalized overall average is 0.33 μg a.i./bee. For the *Phacelia* fields, the amount of tracer per bee was higher than those from the apple orchards, with mean measured values for the 5 trials ranging 6.34 to 35.77 ng/bee, and an overall average of 18.19 ng/bee. The range of averages is equivalent to 0.31 to 1.75 μg a.i./bee when normalized to 1 lb a.i./A and the normalized overall average is 0.89 μg a.i./bee. The tracer was detected at >2.4 μg a.i./bee per 1 lb a.i./A in 3% of individuals foraging in apple orchards and 14% foraging in *Phacelia* fields. The maximum concentration reported in the study was 2.7 μg a.i./bee per 1 lb a.i./A. A detailed description of this study is provided in **Appendix 4**.

3.1.2.2 Alternative method that was considered: T-REX arthropod residue value

The upper-bound pesticide residue value for arthropods used in T-REX is from an analysis completed by EPA that involved compiling empirical data from the scientific literature and registrant-submitted studies. This analysis included data from 14 studies that involved 13 pesticides (primarily organophosphate and carbamate insecticides). These studies measured pesticide residues on various arthropods, including moth and beetle larvae, crickets, grasshoppers, beetles and unidentified arthropods captured in treated areas. The use of the arthropod residue value relies upon measured residues of pesticides in and on arthropods that were located on treated fields at the time the field was sprayed. It is assumed that the residues are predominantly based on direct spray; however, they may

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also incorporate some contact of arthropods with pesticide residues on treated foliage as well as consumption. A detailed description of the analysis completed by EPA to determine an upper-bound arthropod residue value for use in T-REX is available in **Appendix B** of the T-REX user's guide (USEPA 2012*e*).

The extent to which these data are reflective of residues which are likely to occur in or on honey bees is uncertain as honey bees would likely be in flight during pesticide applications as opposed to crawling on soil/leaves. However, the data do represent actual measured residue levels for insects and are expected to be representative of residues on honey bees and other non-*Apis* bees that share the same habitats and behavior. Contact-based toxicity values are expressed on a dose basis (μg a.i./bee). The upper-bound arthropod residue concentration of 94 mg a.i./kg can be converted to a dose of 12 μg a.i./bee (normalized to 1 lb a.i./A or 1.12 kg a.i./ha) by multiplying the concentration by the average body weight of an adult worker bee (0.128 g from Mayer and Johansen 1990).

3.1.2.3 Comparison of Koch and Weisser 1997 and T-REX values to empirical data

The two approaches described above can be evaluated to a limited extent using empirically based measures of pesticides on honey bees foraging in fields sprayed with pesticides. Only two studies have been identified in the literature where chemical concentrations were quantified on bees after foraging in treated fields that were treated foliar spray applications (Hanny and Harvey 1982, Delabie *et al.* 1985). Unfortunately, these authors did not provide maximum residue values; however, they did present mean residues. These values, which are provided in **Table 7**, can be compared to the mean residue values of the T-REX arthropod residue value and Koch and Weisser 1997.

The mean residue value incorporated into T-REX is an order of magnitude above the mean residue values reported by Hanny and Harvey 1982 and a factor of 3 higher than the mean from Delabie *et al.* 1985. The two mean residue values of Koch and Weisser 1997 are higher, but on the same order of magnitude as the residues reported by Hanny and Harvey 1982 and are lower than the mean reported by Delabie *et al.* 1985. Conclusions of the appropriateness of either the T-REX arthropod residue value or Koch and Weisser 1997 for representing upper-bound contact exposures to bees should be limited due to the limited amount of empirical data from other sources; however, the available information suggests that the upper-bound arthropod residue value and the Koch and Weisser 1997 values may

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represent reasonable surrogates for estimating exposures to bees directly sprayed with pesticides. Given that the mean data from Delabie *et al*. 1985 is higher than the means from Koch and Weisser 1997 but not greater than the T-REX arthropod residue mean, the T-REX value may provide a more conservative estimate of exposure.

Table 7. T-REX screen compared to residue data for contact with adult forager bees. Exposure through direct spray on to forager bees measured within 24 hours of application.

Citation	Concentration (mg/kg per 1 lb a.i./A)		Dose (µg a.i./bee per 1 lb a.i./A)		Chemical	Crop
	Upper bound	Mean	Upper bound	Mean		
T-REX (arthropod)	94	65	12	8.3	All	All
Delabie *et al*. 1985	NA	21	NA	2.7	cypermethrin	Oilseed rape
Koch and Weisser 1997	19	6.94	2.7	0.89	sodium-fluorescein*	*Phacelia*
Koch and Weisser 1997	19	2.54	2.0	0.33	sodium-fluorescein*	Apple
Hanny and Harvey 1982	NA	1.19	NA	0.152	carbaryl**	Corn
Hanny and Harvey 1982	NA	0.36	NA	0.045	carbaryl**	Corn

*This chemical is a fluorescent tracer, not a pesticide.
**Two different experiments were conducted using different formulations. The results of the two formulations are presented separately.
NA = not available

3.1.2.4 Assumptions and uncertainties associated with contact exposure methods for foliar applications

There are a limited number of studies available to evaluate the two approaches that could potentially represent the Tier I exposure value for direct spray. Of the two studies that are available, only mean-measured concentrations on bees are reported. Since the Tier I exposure value is intended to represent an upper-bound exposure, not a mean, the utility of the empirically based means in evaluating the upper-bound values is limited. In addition, because there are only two studies available for evaluation purposes, it is unclear how representative these data are related to other locations, crops and field conditions. It is unknown whether or not the data from these two studies are representative of high-end exposures that other bees may receive under different conditions.

According to Atkins *et al.* 1981, when pesticides are applied to crop foliage via spray, the application rate expressed as lb a.i./A is equivalent to the dose received by bees foraging on the treated field, which is expressed as µg a.i./ bee. This conclusion was drawn from comparisons of honey bee mortalities

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observed on treated fields to contact LD_{50} values that were generated in the lab for the same chemicals (N = 65). In the field studies, pesticides were applied to crops in bloom. According to the Atkins' conclusions, an application rate of 1 lb a.i./A or 1.12 kg a.i./ha is equivalent to a contact exposure of 1 μg ai/ bee. The Atkins' value appears to be on the same order of magnitude as the measured chemical residues on bees reported by Koch and Weisser 1997.

3.1.3 Summary of Proposed Tier I Exposure Assessment Method for Foliar Applications

For foliar applications, the proposed method includes two exposure routes: dietary and contact. **Table 8** summarizes the initial residue values expressed in units of μg a.i./bee per 1 lb a.i./A, which are used by the EPA and in units of μg a.i./bee per 1 kg a.i./ha, which are used by other countries, including Canada. As discussed below, these values should be adjusted to account for the application rate of the chemical. For generating RQs, dietary based exposure values can be compared to oral toxicity data for larvae and adult worker bees while contact exposure values can be compared to acute contact toxicity data for adult worker bees.

Table 8. Tier I exposure values used to estimate high end exposures of honey bees to pesticides applied via foliar applications.

Life Stage	Exposure Type	Dose (μg a.i./bee per 1 lb a.i./A)	Dose (μg a.i./bee per 1 kg a.i./ha)
Adult	Diet (nectar + pollen)	32	29
Larvae	Diet (nectar + pollen)	13	12
Adult	Direct spray (T-REX)	12	11
Adult	Direct spray (Koch and Weisser 1997)	2.7	2.1

For dietary exposure, the proposal includes the use of the upper-bound residue for tall grass used in the T-REX model. T-REX is already used for ecological risk assessments of pesticides (for establishing screening-level exposures to terrestrial vertebrates). The current proposal is to use the upper-bound residue for tall grass to represent an upper-bound residue value for pollen and nectar. This value would then be converted to a dietary dose received by adult and larval worker bees using pollen and nectar consumption rates for these two life stages, which are 292 and 120 mg/day, respectively (**Appendix 1**). Therefore, the dietary based exposure value for larvae is 13 μg a.i./bee per 1 lb a.i./A and the value for adults is 32 μg a.i./bee per 1 lb a.i./A. Estimated exposure values can be scaled to an application rate by multiplying by the application rate. In practice, the peak exposure value generated by T-REX accounts for multiple applications and dissipation of the chemical in between applications (based on the foliar dissipation half-life of the chemical). The T-REX user manual (USEPA 2012) provides a detailed explanation of how multiple applications and foliar dissipation are accounted for in the final estimate of exposure.

In order to quantify contact exposures due to direct spray, the proposed upper bound value is 2.7 μg a.i./bee per 1 lb a.i./A, based on data published by Koch and Weisser 1997. As with the dietary exposure, the contact exposure value can be adjusted to account for application rate and multiple applications; however, if the applications are spaced such that it is not reasonable that the same forager bees would be exposed during multiple applications (*i.e.*, >5 days[8]), the direct spray exposure value should be based on only a single application.

3.1.4 Potential options for characterizing exposure (resulting from foliar applications) using available data when Tier I screen is failed

For pesticides that are applied via foliar spray and exceed levels of concern for dietary exposures to bees, it may be possible to refine dietary-based estimates of exposure. This refinement could be accomplished using pesticide-specific measurements on treated foliage (of crops) taken within 24 hours of the application on foliage of crops. The following registrant-submitted magnitude of the residue (MOR) studies may be used for this refinement: crop field trials (OCSPP Guideline 860.1500; USEPA 1996*b*), field rotational crops (OCSPP Guideline 860.1900; USEPA 1996*d*), and irrigated crops (OCSPP Guideline 860.1400; USEPA 1996*e*). In addition, cropped TFD studies (OCSPP guideline 835.6100; USEPA

[8] According to Winston 1987, the lifespan of a forager bee is 4-5 days.

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2008) may also be used. Three limitations exist for this refinement option. First, the MOR studies often do not include measures of pesticide residues on foliage within 24 hours of the application. This is because the studies are in intended to quantify pesticide residues in food items potentially consumed by humans, which would typically be harvested more than 24 hours after the application. However, in situations where residue-decline studies are triggered, day zero data may be available. Second, in order to rely on these chemical-specific data, a sufficient number of residue values from different studies would be required to confidently represent the chemical-specific exposure values. Third, this refinement applies only to dietary-based exposures for bees. If the Tier I methods for both dietary and contact exposures described above yield exposure values that are of concern, then the risk conclusions would not change due to refinement of the dietary-based exposure alone. There is no proposed method for refining exposure estimates for contact.

A potential application of MOR data is explored using MOR data for imidacloprid and unnamed chemical 1, respectively. **Table 9 and Table 10** contain Day 0 residues values in various crops for imidacloprid and unnamed chemical 1, respectively. Compared to the T-REX screening-residue value for tall grass (110 mg/kg per 1 lb a.i./A), the highest residue value for unnamed chemical 1 is within a factor of 2 and the highest residue for imidacloprid is less than a factor of 2. Importantly, a substantial amount of variability in day 0 residues is seen for unnamed chemical 1, both within a crop matrix (but across different field trials) and among crop matrices. Specifically, up to a 4-fold range in day 0 residues can be seen for wheat (forage: 6.8-27.6 mg ai/kg per lb ai/A across two trials in Brazil) and canola (forage: 11.6-42.4 mg ai/kg per lb ai/A across three trials in Australia). The source of this variability is not known, but may reflect subtle differences in pesticide application techniques, climate, crop conditions, and chemical measurements among different residue trials. For these reasons, sufficient day 0 magnitude of residue data on foliage should be available to characterize this variability before such data is used to refine the Tier 1 T-REX foliar residue estimates.

Table 9. Day 0 residue values for imidacloprid (and degradates), and normalized residues to 1 lb a.i./A

Total Application Rate (lb a.i./A)	Day 0 Residues (ppm)	Crop	Matrix	MOR Day 0 Residues normalized to 1 lb Total Application Rate (mg/kg per 1 lb a.i./A)	Country	Citation
0.275	24	Soybean	Hay	87.27	USA	MRID 46785002
0.463	0.19	Succulent beans	Lima beans	0.41	USA	MRID 45046401

Table 10. Unnamed chemical 1 Day 0 residue values, and normalized residues to 1 lb a.i./A

Total Application Rate* (lb a.i./A)	Day 0 Residues** (ppm)	Crop	Matrix	MOR Day 0 Residues normalized to 1 lb Total Application Rate (mg/kg per 1 lb a.i./A)	Country	Citation
0.086	4.3	Barley	Straw	49.9	Australia	Study 1-4
	3.3		Forage	38.3		
0.085	1.3		Straw	15.3	New Zealand	
	1.1		Forage	13.0		
0.086-0.087	1.0-3.7	Canola	Forage	11.6-42.4 (n=3)	Australia	Study 1-5
0.084-0.087	1.8-2.4	Wheat	Straw	20.8-27.7 (n=2)	Australia	Study 1-6
	1.6		Forage	18.5		
0.091-0.093	2.0-3.1		Hay	22.4-33.5 (n=2)	Brazil	
0.085-0.094	0.6-2.6		Forage	6.8-27.6 (n=2)		
0.087	1.8		Straw	20.8	New Zealand	

* sum of two applications
** residue samples collected on day of second application

In addition to comparing day 0 MOR data to the T-REX foliar application Tier 1 screening value, it is also instructive to compare MOR data to available data on residues in pollen and nectar for the same chemical (**Table 11**). When the MOR Day 0 studies are compared to available pollen and nectar data for unnamed chemical 1, the highest Day 0 MOR residue (49.9 mg ai/kg per lb ai/A) is slightly higher than the highest measured concentration of the chemical in pollen (31.3 mg ai/kg per lb ai/A), suggesting that foliar residues may be a reasonable surrogate for residues in pollen (at least on day 0). While the higher day 0 residue values measured in the MOR studies exceed residues measured in pollen for unnamed chemical 1, many MOR day 0 values are below the maximum residue measured in pollen. Again, this indicates the importance of obtaining sufficient MOR data to characterize the variability in residue values among different crops, plant matrices and fields. Otherwise, the available MOR Day 0 value may not be protective for pollen. When compared to the highest concentration of unnamed chemical 1 in nectar (2.2 mg ai/kg per lb ai/A), the MOR Day 0 values are all above the day 0 residue value for nectar

(overall range: 6.8-49.9 mg ai/kg per lb ai/A). This suggests that MOR day 0 residue data may be more conservative (i.e., more protective) in relation to residues in nectar compared to pollen, at least for unnamed chemical 1.

Table 11. Unnamed chemical 1 pollen and nectar data compared to magnitude of residue Day 0 residues.

Citation	Crop Matrix	Concentration (mg/kg-plant per 1 lb a.i./A)
Study 1-1	Cotton – pollen[1]	4.2-31.3
	Cotton-nectar[1]	<0.22-0.83
	Phacelia –pollen[2]	13.5-18.9
Study 1-2	*Phacelia* – nectar[2]	2.1-2.2
Study 1-6	Wheat - forage	6.8
Study 1-4	Barley - straw	49.9

[1] values represent range in maximum measured residues in forager-collected pollen and nectar on day 0 from 4 tunnel trials with cotton.
[2] values represent range in maximum measured residues in pollen collected from traps and forager-collected nectar from 2 tunnel trials with *Phacelia*.

In summary, this example indicates that day 0 residue measurements from MOR studies have the capacity to provide some additional refinement of the Tier 1 pollen and nectar screening value from T-REX described earlier. However, use of day 0 MOR data is subject to uncertainty related to the limited available data from which to characterize variability in foliar residues among crops. Furthermore, use of chemical-specific foliar residue data still does not address uncertainties when extrapolating foliar residues to those expected in pollen and nectar.

3.2 Quantifying Exposure Resulting from Soil Applications

For soil treatments, it is assumed that bees will be exposed via dietary consumption of pollen and nectar that are contaminated as a result of systemic transport of pesticides from soil. For these application types, it is assumed that honey bees will not be directly sprayed because they are not expected to be present on the surface of the soil. The proposed method for estimating dietary exposures to bees resulting from soil treatments is based on an empirically based model developed by Briggs *et al.* 1982 and 1983, with modifications (referred to in this white paper as "the Briggs' Model"). This model relates the Log K_{ow} of a chemical to its concentration in plant shoots, which (like the T-REX approach for foliar spray applications) can be used as a surrogate for concentrations in nectar and in pollen. In comparison to the dietary-based exposure values proposed for foliar spray applications (*i.e.*, based on the tall grass upper-bound value), the Briggs' model generates exposure values that are two orders of magnitude lower. Also discussed in the section below is another method that was considered in the development of the Tier I exposure method for soil applications: the EPPO screening value of 1 mg a.i./kg in pollen and nectar of treated crops. EPPO's screening value is based on data compiled by Alix *et al.* 2009, including pesticide residues measured in different plant parts (leaves, fruit, green part, inflorescence, whole plant, and grain) following applications to soil or seed treatments. Evaluations of both the Briggs' Model and the 1 mg a.i./kg screening value are described and evaluated below. These approaches are evaluated using empirical measurements of pesticides in pollen and nectar of crops that received soil treatments of the pesticides. These data are provided in **Table 12** and summaries of the studies used to generate the empirical data are provided for reference purposes in **Appendices 3 and 4**.

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Table 12. Measured concentrations of pesticides in pollen and nectar of crops treated with pesticides via soil applications. Concentration units are unnormalized (*i.e.*, expressed as mg a.i./kg) and normalized to application rate (*i.e.*, expressed as mg a.i./kg per 1 lb a.i./A).

Chemical	Citation	Crop	Application rate (lb a.i./A)	Concentration (mg a.i./kg)		Normalized Concentration (mg a.i./kg per 1 lb a.i./A)	
				Nectar	Pollen	Nectar	Pollen
Dimethoate	Lord *et al.* 1968	Fuchsia	17	4.82	NA	0.283	NA
Dimethoate	Lord *et al.* 1968	Nasturtium	17	1.26	NA	0.0726	NA
Dinotefruan*	Dively and Kamel 2012	Pumpkin	0.270	0.0154	0.0812	0.057	0.301
Dinotefruan*	Dively and Kamel 2012	Pumpkin	0.270	0.0109	0.0269	0.040	0.100
Imidacloprid	Stoner and Eitzer 2012	Squash	0.344	0.014	0.028	0.041	0.081
Imidacloprid*	Beedle and Harbin 2011	Cotton	0.063	0.066	NA	1.1	NA
Imidacloprid*	Freeseman and Harbin 2011	Tomato	0.25	NA	0.054	NA	0.22
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.027	0.0007	0.0094	0.026	0.351
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.251	0.0113	0.0567	0.045	0.226
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.377	0.0178	0.109	0.047	0.288
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.377	0.0231	0.129	0.061	0.341
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.027	ND	ND	ND	ND
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.251	0.0067	0.0239	0.027	0.0953
Imidacloprid*	Dively and Kamel 2012	Pumpkin	0.377	0.016	0.0440	0.042	0.117
Oxamyl	Dively and Kamel 2012	Pumpkin	0.125	ND	ND	ND	ND
Oxamyl	Dively and Kamel 2012	Pumpkin	0.125	ND	ND	ND	ND
Thiamethoxam	Stoner and Eitzer 2012	Squash	0.127	0.020	0.014	0.16	0.11
Thiamethoxam*	Dively and Kamel 2012	Pumpkin	0.171	0.0186	0.132	0.109	0.768
Thiamethoxam*	Dively and Kamel 2012	Pumpkin	0.171	0.0151	0.0420	0.088	0.245
Unnamed chem. #2	Study 2-4	Melon	0.089	0.026	0.012	0.29	0.14

NA = not available
ND = not detected
*represents total residues (*i.e.*, parent and degradates)

3.2.1 Proposed method for estimating exposures from soil applications: Briggs' Model

3.2.1.1 Method Description and Evaluation

In 2006, the United Kingdom's Environment Agency reviewed several models that predict chemical concentrations in plants that result from uptake from soil (Environment Agency 2006). Models that were reviewed included those published by Chiou *et al.* 2001, Hung and MacKay 1997, Briggs *et al.* 1982 and 1983 with additions by Ryan *et al.* 1988, Topp *et al.* 1986, Trapp and Matthies 1995 and Travis and Arms 1988. This review involved comparisons of model-estimated concentrations of chemicals to five sets of empirical data from different studies. These datasets included 13 chemicals with the Log K_{ow} values ranging 0.18-6.5. Of the six models that were evaluated, the model by Briggs *et al.* 1982 and 1983 with modification by Ryan *et al.* 1988 estimated chemical concentrations in shoots that were closest to empirical data, with estimated values being more conservative, and yet within an order of magnitude of empirical values. The model by Briggs *et al.* 1982 and 1983 is also of interest because it is incorporated into the Pesticide Root Zone Model (PRZM; Carosusel *et al.*, 2006). Although PRZM is used in this paper to estimate pesticide runoff from treated fields, the plant uptake portion of the model is not incorporated into the calculations. The model developed by Briggs *et al.* 1982 andd 1983 and as revised by Ryan *et al.* 1988 is considered further as a potential Tier I screening method for estimating pesticide exposures to honey bees feeding upon crops where the soil was treated with pesticides.

The Briggs' model is based on an empirical relationship between measured K_{ow} values and stem concentration factors (SCFs). The SCFs are ratios of concentrations in plant stems (*i.e.*, shoots) to measured concentrations in the aqueous medium from which plant roots uptake the chemical. They are calculated using empirically based transpiration stream concentration factors (TSCFs), which are a ratio of the concentration of the chemical in the xylem to the concentration in the aqueous medium. The relationship between K_{ow} and TSCF, was derived using data for 17 pesticides, including carbamate insecticides and phenylurea herbicides with Log K_{ow} values ranging from -0.57 to 4.6. All of the pesticides included in the dataset used to develop the Briggs' model were non-ionic organic chemicals. The regression is based on empirically based K_{ow} values and measures of concentrations in steams and aqueous medium (**Equation 1**).

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Equation 1

$$SCF = \left[10^{(0.95*LogKow-2.05)} + 0.82\right] * TSCF$$

Where: $TSCF = \left[0.784 * 10^{-0.434*(LogKow-1.78)^2/2.44}\right]$

The Briggs' approach was modified by Ryan *et al.* 1988 by adding an equilibrium partitioning component that allows for estimation of pesticide concentrations from soil (**Equation 2**), rather than aqueous medium as was used by Briggs. **Equation 2**, which is the Briggs' model with the modification by Ryan *et al.* 1988, can be rearranged to estimate the pesticide concentration in plant shoots using three input parameters: K_{ow}, K_{oc} and application rate. Note that if K_{oc} is not available or appropriate for a chemical, the K_d can be used by replacing the following portion of **Equation 2:** $K_{oc} * f_{oc}$.

Equation 2

$$SCF' = \frac{C_{stem}}{C_{soil}} = \left[10^{(0.95*LogKow-2.05)} + 0.82\right] * TSCF * \left[\frac{\rho}{\theta + \rho * Koc * foc}\right]$$

Where:

Where: SCF' = stem concentration factor based on uptake from soil
C_{stem} = concentration in stems (μg a.i./g plant)
C_{soil} = concentration in soil (μg a.i./g soil)
f_{oc} = fraction of organic carbon in soil
θ = soil-water content by volume (cm^3/cm^3)
ρ = soil bulk density (g-dw/cm^3)
K_{oc} = soil organic carbon-water partitioning coefficient (cm^3/g-oc or L/kg-oc)

The Briggs' model with the modifications by Ryan *et al.* 1988 is evaluated here by comparing estimated concentrations in stems to empirical data available for pesticide concentrations in nectar and pollen in studies where pesticides were applied to soil and transported into these matrices. Data are available for five chemicals, including dimethoate (Lord *et al.* 1968), dinotefuran, imidacloprid, thiamethoxam (Dively and Kamel 2012), and unnamed chemical #2 (**Table 13**). For these chemicals, K_{ow} and K_{oc} data were collected from registrant-submitted data. For the parameters in **Equation 2** that define the properties of the soil, conservative values were chosen to maximize the concentration in soil pore water and thus maximize the amount of chemical available for uptake into stems. Values were chosen to be consistent with standard scenarios used to run PRZM. For the fraction of organic carbon in soil (f_{oc}), a value of 0.01 is used. A value of 1.5 g-dw/cm^3 is selected to represent bulk density (ρ). Soil water content (θ) is set to 0.2 cm^3/cm^3. **Equation 2** can be used to calculate the concentration of a chemical in stems using the

parameter values above and the application rate. For consistency among the reviewed methods, 1 lb a.i./A (1.12 kg a.i./ha) is used as an application rate. If it is assumed that this application rate is homogenously distributed throughout the top 6 inches (15 cm) of the treated soil (based on the assumption that the majority of the pesticide will remain in this portion of the soil), this rate is equivalent to 0.50 μg a.i./g-soil (using the bulk density).

The resulting estimated values of pesticides are generally within an order of magnitude of empirical data of the same pesticides in nectar and pollen (**Table 13**). This indicates that the Briggs' model with modifications by Ryan *et al*. 1988 may provide reasonable estimates of pesticides in nectar and pollen due to systemic transport after applications to soil. For the Tier I screen, which is intended to be conservative, this approach is limited because the estimated values are not always higher than the empirical data. This is not surprising since the relationship between K_{ow} and SCF that was derived by Briggs *et al*. 1982 and 1983 is based on a median estimate of the SCF value, not an upper bound.

Table 13. Comparison of estimated concentrations of pesticides in plant stems (based on Equation 2) and empirical data measurements in nectar and pollen. Concentration units are mg a.i./kg-plant matrix normalized to 1 lb a.i./A.

Chemical	Log K_{ow}	K_{oc}	Estimated concentration in stems (mg a.i./kg per 1 lb a.i./A)	Empirical concentrations (mg a.i./kg per 1 lb a.i./A)	
				Nectar	Pollen
Dimethoate	0.78	30	0.52	0.0726-0.283	NA
Dinotefruan*	-0.55	6	0.18	0.040-0.057	0.100-0.301
Imidacloprid*	0.57	178	0.10	0.026-1.1	0.081-0.351
Thiamethoxam*	-0.13	33.1	0.16	0.088-0.16	0.11-0.768
Unnamed chem. #2	1.94	241	0.039	0.29	0.14

*Empirical data include parent and degradates.
**Residues measured in tomato anthers
NA = not available

In order to use of the Briggs' model for deriving more conservative estimates of concentrations of pesticides in plants, the original relationship between TSCF and Log K_{ow} was recalculated. This was done using the SAS® version 9.2 (SAS Institute, Cary, NC) nonlinear regression procedure (PROC NLIN) with the Gauss method of optimization to solve for the three unknown parameters relevant to the equation. This method differs from the original approach reported by Briggs *et al.* 1982 and 1983 in that they did not use the non-linear regression method of analysis. In addition, the recalculated relationship includes all of the TSCF values reported by Briggs *et al.* 1982 and 1983 while the original equation excluded one value that was assumed by the authors to be an outlier. In addition to the mean estimates of TSCF values, the current analysis involved the calculation of the 95th percentile upper-bound estimates of TSCF values (**Figure 9**).[9] The median and 95th percentile estimated TSCFs (based on Log K_{ow}) are provided in **Appendix 5**. The 95th percentile TSCF values can be used in **Equation 2** to calculate upper-bound estimates of pesticide concentrations in stems. The resulting estimates are conservative and are generally within an order of magnitude of empirical estimates of pesticides in nectar and pollen (**Table 14).**

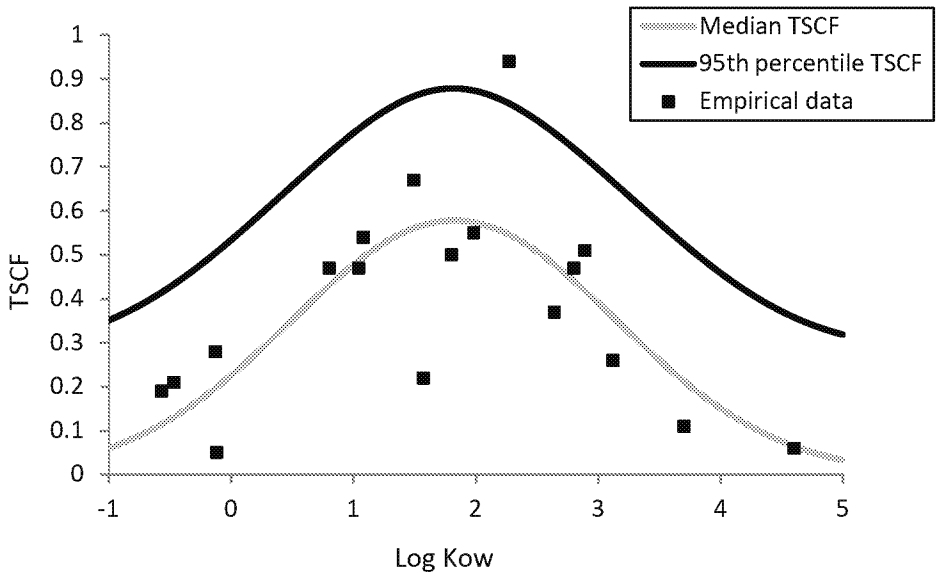

Figure 9. Median and 95th percentile upper-bound TSCF data fit to Briggs *et al.* (1982 and 1983) empirically-based TSCF data for 18 pesticides.

[9] It should be noted that in the original TSCF equation reported by Briggs *et al.* (1982) was based on n = 17 because one "outlier" was excluded. The recalculated median and 95th percentile TSCF values are based on all of the empirical data reported by Briggs *et al.*

Table 14. Comparison of estimated concentrations of pesticides in plant stems (based on Equation 2 and 95th percentile TSCF) and empirical data measurements in nectar and pollen. Concentration units are mg a.i./kg-plant matrix normalized to 1 lb a.i./A (1.12 kg a.i./ha).

Chemical	Log K_{ow}	K_{oc}	Upper-bound TSCF	Estimated concentration in stems (mg a.i./kg per 1 lb a.i./A)	Empirical concentrations (mg a.i./kg per 1 lb a.i./A)	
					Nectar	Pollen
Dimethoate	0.78	30	0.726615	1.46	0.0726-0.283	NA
Dinotefuran*	-0.55	6	0.417797	1.78	0.040-0.057	0.100-0.301
Imidacloprid*	0.57	178	0.67502	0.30	0.026-1.1	0.081-0.351
Thiamethoxam*	-0.13	33.1	0.503762	0.90	0.088-0.16	0.11-0.768
Unnamed chem. #2	1.94	241	0.875448	0.026	0.29	0.14

*Empirical data include parent and degradates.
NA = not available

3.2.1.2 Assumptions and Uncertainties

There are five notable limitations to using the modified Briggs' model approach. The first is that this methodology is based on empirical data from only one type of plant. Uptake may be different for different types of plants (Ryan *et al.* 1988); however, the species used by Briggs *et al.* 1982 (*i.e.,* barley) is representative of a crop species that is likely grown on soils where pesticides are applied.

The second limitation is that the data set used to derive **Equation 1** is based on a limited number of chemicals that represent only two classes of pesticides. Despite this limitation, the pesticides that were used in this approach are systemic and cover a wide range of Log K_{ow} values (*i.e.,* range is -0.57-4.6), leading to an approach that is applicable to a wide range of pesticides with the potential to be systemically transported by plants.

The third limitation is that this approach is based on data from non-ionic organic chemicals and may have limited utility for ionic chemicals that whose transport may not be predicted well using K_{ow} and K_{oc}. Generally, compounds that are lipophilic and non-polar will cross plant cell membranes more readily than charged compounds do. Nevertheless, charged compounds may enter a cell via active transport, which requires energy, or passive transport, which is a diffusive process (Trapp 2004). Thus, the ion trapping effect of weak electrolytes (*i.e.,* most pesticides, particularly herbicides) leads to movement through the phloem and equations used to model this process are based on two primary concepts: weak

acid and intermediate permeability theories (Trapp 2004) which are represented in the model by acid strength (pKa) and polarity/lipophilicity (K_{ow}), respectively (Bromilow *et al.* 1990).

The fourth limitation of the Briggs' model is that it is based on passive transport of chemicals into xylem of barley. This approach does not directly estimate pesticide concentrations in plants that are the result of phloem transport. One possible assumption that could be made in applying this model for estimating pesticide concentrations in plants would be to assume that chemical transport via phloem is equivalent to that of transport via xylem. An alternative approach would be to use different models for neutral and ionic compounds, such as those described by Briggs *et al.* 1987, Kleier 1988 and Trapp *et al.* 1995.

The fifth limitation involves the use of estimated pesticide concentrations in vegetative plant matrix (*i.e.*, shoots) as a surrogate for nectar and pollen. At this point, the relative concentrations of a chemical in pollen and nectar to plant shoots is unknown; however, estimated concentrations of a select number of pesticides in shoots appear to be protective of values in pollen and nectar (**Table 14**). Data reported by Dively and Kamel 2012 indicate that for soil drench, transplant dip and drip applications of imidacloprid, dinotefuran and thiamethoxam, pesticide (plus degradate) concentrations in foliage were higher but on the same order of magnitude compared to concentrations in pollen and an order of magnitude higher than concentrations in nectar than concentrations in nectar.

3.2.2 Alternative method that was considered: EPPO's 1 ppm default value

3.2.2.1 Method Description and Evaluation

In the screening-level pollinator exposure assessment for pesticides applied via soil treatments described by EPPO, if pesticide-specific residues in pollen and nectar are not available, a screening value of 1 mg ai/kg plant matrix is the assumed exposure (EPPO, 2010). This screening value was considered when developing the proposed method to represent a Tier I exposure value for soil applications. EPPO's screening value of 1 mg a.i./kg is based on the maximum value from data compiled by Alix *et al.* 2009, including pesticide residues measured in different plant parts (leaves, fruit, green part, inflorescence, whole plant, and grain) following applications to soil or seed treatments.

When the 1 mg a.i./kg default value is compared to maximum residue values from available studies where pesticides were applied to soil, all residue values for pollen are below the screening value (**Table 12**). There is one residue value (from Lord *et al.* 1968) for nectar that exceeds the screen by a factor of

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almost 5 (**Table 12**); however, this value was from a study where the application rate of dimethoate was equivalent to 17 lb a.i./A, which is much higher than currently registered application rates of this chemical (and most other insecticides) in North America. With the exception of the dimethoate data, all of the measured concentrations of pesticides (values not normalized) in pollen and nectar of plants (receiving soil applications) are 2 orders of magnitude below the 1 mg a.i./kg screening value. Based on this information, it appears that the 1 mg a.i./kg default value is a reasonably conservative estimate of exposure for pollen and nectar of crops treated with pesticides from soil applications.

3.2.2.2 Assumptions and Uncertainties

The proposed use of the EPPO screening-level exposure value of 1 mg a.i./kg for pollen and nectar of seed treated crops does not account for differences in application rate. There is uncertainty in not accounting for the mass of the chemical applied since it is expected that the magnitude of the pesticide concentration in the plant will be influenced by the magnitude of the application. This is illustrated by the dimethoate data published by Lord *et al.* (1968), where a high application rate (*i.e.*, 17 lb a.i./A) resulted in concentrations in nectar that exceeded 1 mg a.i./kg. Although this application rate is not relevant to current registrations for dimethoate or most insecticides, some pesticides are currently registered for soil applications at similar rates. In addition, this approach does not account for physical-chemical properties of a pesticide that may influence its potential systemic transport throughout the plant and into the pollen and nectar. For example, a chemical with low mobility (high K_{oc}, high K_{ow}) may be expected have lower concentrations in nectar when compared to a chemical with high mobility (*i.e.*, low K_{oc}, low K_{ow}).

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3.2.3 Summary of Tier I Method for Soil Applications

Despite the limitations discussed above, both the EPPO 1 mg a.i./kg screening and the Briggs' model modified by Ryan *et al*. 1988 and with the further modifications proposed in this white paper (*i.e.,* with 95th percentile TSCF values) appear to be useful tools for generating reasonably conservative estimates of pesticide concentrations in plants after systemic transport from soil. These approaches are designed to estimate pesticide concentrations in plants after translocation from soil; therefore, they could potentially be used for pesticides that are applied directly to the soil. The approaches are consistent with a screening-level assessment because they are both simple and efficient, and are empirically based on data for pesticides. The Briggs' model has the advantage that it relies upon some basis physical-chemical properties of pesticides and also accounts for the mass applied to the soil; therefore, the Briggs' model is proposed for estimating pesticide concentrations in pollen and nectar of crops as a result of systemic transport of pesticides following applications to soil.

In using the Briggs' model, the proposed approach is to use **Equation 2**, with the 95th percentile TSCF value that is specific to an assessed pesticide's Log K_{ow} (**Appendix 5**). It is assumed that the resulting value is equivalent to pesticide concentrations in pollen and nectar of crops receiving soil treatments of the pesticide. The estimated concentration in pollen and nectar would be converted to dietary-based exposures for adult and larval bees using the consumption rates for pollen and nectar as discussed in **Appendix 1** (*i.e.,* 292 and 120 mg/day, respectively).

In the Tier I approach, it is assumed that all chemicals may be systemically transported. This assumption may be limited based on Log K_{ow} (Ryan *et al*. 1988). Therefore, it could be limited to the bounds of the Log K_{ow} values used in the empirical data set generated by the Briggs' model (*i.e.*, for chemicals with Log K_{ow}<5). Whether a chemical is transported systemically in plants could potentially be confirmed using empirical data submitted to EPA (*e.g.,* plant metabolism studies); however, it would be up to pesticide registrants to submit sufficient data to demonstrate that a pesticide is not systemic.

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3.3 Potential options for characterizing exposure using available data when Soil Treatment Tier I screen is failed

In the development of the Tier I method for assessing honey bee dietary exposures to pesticides resulting from soil applications, the potential utility of empirical data that are required by the EPA for pesticide registrations was explored. Five sets of data were investigated, including: terrestrial field dissipation (TFD) studies with cropped fields (OCSPP guideline 835.6100; USEPA 2008), crop field trials (OCSPP guideline 860.1500; USEPA 1996*a*), nature of the residue in plants (OSCP Guideline 860.1300; USEPA 1996*b*) and confined as well as field accumulation in rotational crop studies, *i.e.*, OCSPP guideline 860.1850 (USEPA 1996*c*) and OSCP Guideline 860.1900 (USEPA 1996*d*). A basic description of each of these studies is provided below along with their limitations for use in the Tier I screen. In summary, none of these studies are specifically designed to measure high-end pesticide concentrations in pollen and nectar at times when bees may be exposed. With some modification to the study designs, some of these studies may be used to generate chemical-specific measures of pesticides in nectar and pollen. This is a potential consideration for refined exposure assessments for chemicals that do not pass the Tier I screen. Although it was determined that these studies are not appropriate for development of the exposure method for the Tier I screen, some of these studies may be useful for characterization of potential exposures for pesticides that fail the screen. A discussion of the potential utility of these studies for characterizing exposures is provided below.

3.3.1 Terrestrial Field Dissipation Studies for a Cropped Field

TFD studies are required for pesticide active ingredients with outdoor use patterns. The purpose of a TFD study is to provide data on the dissipation of a pesticide under actual use patterns which are expected to account for multiple transport (*e.g.,* volatilization, leaching) and degradation processes (*e.g.,* metabolism, hydrolysis). TFD studies report the amounts of the applied pesticide active ingredient (and its degradates) in samples collected from different environmental compartments (*e.g.,* soil, air and vegetation) over time. For the most part, submitted TFD studies involve bare ground applications of pesticides (*i.e.*, no crop is present on the study site); therefore, the existing studies that have been submitted to support pesticide registrations are not useful for estimating pesticide concentrations in plants as a result of systemic transport from soil. The existing guideline (OCSPP 835.6100; USEPA 2008) does include guidance for registrants to conduct the study with a standing crop if it is believe that

uptake may be an important route of removal from the field. However, the sampling design for the cropped field studies does not provide an adequate upper-bound estimate of pesticide exposure.

For example, one of the few TFD studies submitted that was conducted using unnamed chemical #1 applied as three foliar sprays to spinach measured residues in whole spinach plants 8, 11, and 14 days after the initial application (3 days after 2nd application, and 3 days after 3rd application). Residues were also measured in two rotational crops with a 77-day plant-back (cantaloupe) and a 245-day plant-back (wheat). Although these data may provide some information about the relative persistence of a chemical and provide information on the systemic nature of a chemical, they are not necessarily designed to obtain upper-bound estimates of pesticide concentrations in plants. In the future, TFD studies could be designed on a chemical-by-chemical basis to provide empirical measurements of pesticide residues in plant foliage (that can be used as a surrogate for nectar and pollen) or designed to directly measure pesticide residues in pollen and nectar. Specifically, the TFD study is conducted for at least 365 days (one year), where for regions that are productive year round (*e.g.*, California) multiple crops in rotation (see crop rotational studies below) could be studied and residues measured in plant matrices relevant for bee exposure.

3.3.1.1 Crop Field Trials (magnitude of residue studies)

Crop field trials, which are also referred to as magnitude of residue (MOR) studies, measure residue levels in or on raw agricultural commodities (RACs; *e.g.*, fruit, lettuce leaves, grain) relative to actual application methodologies and practices in the field. The pesticide is applied under conditions expected to result in the highest residues on the RACs, and sampling techniques are representative of field growing and harvesting conditions for the crop, and crops are harvested at commercial maturity of the RAC. The residues are used to evaluate the potential dietary exposure for human health risk assessments, and to set tolerances for the chemical on different crops. For the Tier I exposure assessment method, there are several limitations that prevent the use of empirical data from MOR studies. The sampling methodologies that are used the residues estimated for the RACs from the crop field trials do not necessarily generate conservative exposure values for dietary items relevant to bees; however, this is not a defect of the study design for its intended purpose, which is to derive measurements of pesticide concentrations that may be present on food items for human consumption collected from fields treated with pesticides. Measurements are taken days, weeks, and sometimes months (for soil treatments) after application; therefore, the amount of pesticide residue has decreased

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over time (*i.e.,* lost to degradation to breakdown products), and may not be representative of the residues during time of bloom when flowers are present and honey bees may be foraging on the crop.

3.3.1.2 Nature of Residue in Plants (Plant metabolism studies)

The plant metabolism studies are required whenever a pesticide use is determined to be a food use in order to quantify the metabolic fate of the parent active ingredient using radioactive compounds. These studies are intended to characterize the distribution of pesticide residues (including parent and degradates) in treated crops. Metabolism studies are versatile in that residues may be estimated from portions of the plant considered edible to humans (*e.g.,* lettuce leaves, apple fruits, tomato pulp and peel), as well as inedible portions of the plant such as cotton bolls, apple tree leaves, tomato stems, and roots. Despite this versatility, most studies are designed to capture pre-harvest applications in order to measure residues in fruit and mature plant parts relevant to human consumption through diet. Therefore, the sampling strategy (*i.e.*, the timing of sampling) of this study is not designed to generate conservative exposure values at times when residues in plants may be highest for bees and on dietary items that are relevant to bees.

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3.3.1.3 Accumulation in Rotational Crops

Submitted studies on pesticide accumulation in rotational crops, both confined accumulation and field accumulation (OSCP Guideline 860.1500; USEPA 1996*a*) are conditionally required when a pesticide is proposed for rotational crop use (*e.g.,* any field vegetable crop or when it is reasonably foreseeable that any food or feed crop may be planted after harvest of the treated crop). These studies are designed to determine the nature and amount of pesticide residues that are taken up by rotational food crops. A confined rotational crop study is first conducted using small plots and radiolabeled pure active ingredient. Based on the results of the confined study, a field rotational crop study may be required. The field rotational crop study is conducted using the end- use product and is designed to determine whether residues occur in rotational crops grown under actual field conditions. Both rotational crop studies are conducted on food crops that belong to three rotated crop categories: 1) small grain 2) leafy vegetable or soybean; and, 3) root crop. Following application of the pesticide to bare soil, untreated rotational crops are planted following three soil aging intervals (30, 120, and 365 days), or plant-back intervals (PBI)[10]. Residues are quantified plant tissues considered relevant to human and livestock consumption. Based on the results from the rotational crop studies, the Agency may establish appropriate crop rotation restrictions (time from application to planting of rotational crop) and determine the need for tolerances on the rotated crops.

Although the rotational crop studies are designed to address human health risk assessment needs, the Agency believes these studies also have utility in assessing risks to insect pollinators (honeybees). Specifically, results from rotational crop studies can be used in assessing the extent to which a pesticide is systemic. Whether a compound is systemic is an important consideration in assessing the potential of pesticide to accumulate in pollen and nectar. Although in many cases the systemic nature of a pesticide is known based on the nature of its proposed uses, its chemical class, or other information submitted by the registrant; in other cases, direct knowledge of whether a compound is systemic may not be known. Because the pesticide in question is applied to bare soil prior to planting the rotated crops, the residues quantified in plant tissue are an indication of systemically-transported chemical, since chemical transport from the soil into the plant is required. In addition to the magnitude of the parent pesticide, the amount of pesticide metabolites is also quantified by these studies, which is equally relevant to pollinator risk assessment. An illustration of results from confined rotational crop studies is provided in

[10] The PBIs reflect various potential rotational crop circumstances, such as crop failure (30 days), immediate crop rotation following primary crop harvest (120 days) and annual crop rotation (365 days).

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Table 15 and **Table 16** for two pesticides: clothianidin and unnamed "chemical 1". Had the systemic nature of these pesticides not already been known, such information could have been considered by the Agency as an indication of systemic transport in plants and potential transport to pollen and nectar.

Another way in which results from rotational crop studies can be used in pollinator risk assessment pertains to the extent that seasonal pesticide "carry over" becomes an important consideration in exposure assessment. If pesticide residues of concern are found to accumulate in rotated crops at toxicologically-relevant levels, then the pollinator exposure assessment may need to account for pesticide accumulation by plants (pollen and nectar) over multiple growing seasons. In these situations, adjustment of exposure model input parameters may be required to account for prior accumulated pesticide residues and field study designs may need to be altered to include multiple seasons of pesticide application. Data presented in **Table 15** indicate that soil-applied clothianidin can persist and accumulate in rotational food crops for periods of one year, although residues decline over time and the lowest residues are found in seed (grain). For unnamed "chemical 1," rotational crop data indicate that the parent chemical may persist and accumulation in some portions of rotational crops, but the primary metabolite persists and accumulates in rotational crops at higher levels (**Table 16**). Importantly, in order to evaluate the need to address multi-season carryover in the context of pollinator assessment for either of these two chemicals, residues would need to be considered in the context of potential exposure and toxicity to pollinators.

Although data from rotational crop studies can have utility in pollinator risk assessment, a number of limitations are apparent which require careful consideration. First, these studies do not quantify residues in pollen and nectar which are the primary routes of oral exposure to honeybees. Thus, there is considerable uncertainty in the extent to which residues in various plant tissues can be used to represent potential residue in pollen and nectar. Second, in terms of evaluating the extent to which a compound is systemic, the shortest PBI used in typical rotational crop studies is 30 days. Therefore, the extent to which less persistent pesticides (*e.g.*, soil half-life <10 days) are systemic in plants may not be readily identified. It is further noted that results from rotational crop studies are likely to be most applicable to pesticides which are applied via soil application, as the nature of systemic transport in plants can depend on the application method (foliar, soil, seed) and point of contact with the plant (roots, stem, leaves).

Table 15. Sample parent compound residue data for a confined rotational study (860.1850, MRID 45422618) using soluble concentrate formulation of clothianidin. A single application of 0.293 lbs a.i./A, applied directly to bare soil prior to planting rotation.

Plant back interval (days)	Turnip		Swiss Chard	Wheat			
	Top	Root		Forage	Hay	Straw	Grain
29	0.114	0.006	0.054	0.137	0.091	0.327	0.003
153	0.067	0.004	0.056	0.126	0.057	0.136	<0.002
314	0.023	0.002	0.025	0.072	0.046	0.089	<0.002

Table 16. Sample residue data for a confined rotational study (860.1850) of unnamed chemical 1 applied to directly to bare soil using a single application of 0.535 lbs a.i./A.

Plant back interval (days)	Mature Radish		Mature Lettuce	Wheat			
	Top	Root	Leaf	Forage	Hay	Straw	Grain
Parent							
30	0.042	<0.001	0.013	0.010	0.004	0.020	<0.001
120	0.009	<0.001	<0.001	<0.001	0.018	0.106	<0.001
365	0.021	<0.001	0.007	<0.001	<0.001	0.009	<0.001
Primary Metabolite							
30	0.773	0.135	0.365	0.835	0.658	0.952	0.046
120	0.329	0.077	0.286	0.183	1.076	1.452	0.050
365	0.610	0.047	0.176	0.138	0.455	0.330	0.012

3.4 Quantifying Exposure Resulting from Seed Treatments

3.4.1 Method Description and Evaluation

The proposed Tier I exposure method for seed treatments is based on the EPPO 2010 screening value of 1 mg a.i./kg in pollen and nectar of plants to which seed treatments were made. For these application types, it is assumed that bees will only be exposed through the diet. A discussion of other potential exposure routes, such as dust is provided in the section below, but is not quantified in the Tier I method for assessing pesticide exposures to bees from seed treatments. In the Tier I approach, it is assumed that all pesticides that are applied to seeds are systemic, and therefore can be transported into pollen and nectar that may be consumed by honey bees. This approach may be used for all pesticides that are applied via seed treatment, by assuming that the upper-bound exposure value for pollen and nectar is 1 mg a.i./kg. This value can be multiplied by nectar and pollen consumption for adult and larval worker bees to determine the upper-bound doses potentially received by bees. This method can be applied to all chemicals that are applied to seeds, with no need for adjustment based on application rate or chemical properties.

The effectiveness of the EPPO's screen was evaluated using empirical data describing pesticide concentrations in pollen and nectar of crops whose seeds were treated with the pesticide. Data were located in nine different studies, representing six different chemicals, the majority of which were neonicotinoid insecticides, which are known to be systemic compounds (**Table 17**). The highest residue value measured in pollen and nectar samples was 0.036 mg a.i./kg, which a factor of 28 lower than the EPPO screen (*i.e.*, 1.0 mg a.i./kg). As a result, EPPO's screening value of 1 mg a.i./kg appears to be a reasonably conservative estimate of pesticide residues in pollen and nectar of crops to which pesticides are applied via seed treatment.

Table 17. Measured concentrations of pesticides in pollen and nectar of plants where seeds were treated. Data are not normalized to app rate.

Chemical	Rate (mg a.i./seed)	Crop	Measured concentration (mg a.i./kg)		Citation
			Pollen	Nectar	
Imidacloprid	1	Sunflower	0.036*	NA	Laurent and Rathahao 2005
Imidacloprid	1	Corn	0.018*	NA	Bonmatin *et al.* 2005b
Imidacloprid	0.7	Sunflower	0.011*	NA	Bonmatin *et al.* 2005a
Imidacloprid	0.7	Sunflower	0.0039**	0.0019**	Schmuck *et al.* 2001
Clothianidin	1.25	Corn	0.0039**	NA	Krupke *et al.* 2012
Clothianidin	1.25	Corn	0.0039**	NA	Staedtler 2009
Clothianidin	1.25	Corn	0.0039**	NA	Staedtler 2009
Clothianidin	1.25	Corn	0.0034**	NA	Staedtler 2009
Metalaxyl	NA	Corn	0.0031**	NA	Krupke *et al.* 2012
Clothianidin	1.25	Corn	0.0029**	NA	Staedtler 2009
Clothianidin	1.25	Corn	0.0029**	NA	Staedtler 2009
Clothianidin	NA	Oilseed rape	<0.001+	0.003	Wallner 2009
Clothianidin	NA	Canola	0.00259*	0.00224*	Cutler *et al.* 2007
Thiamethoxam	NA	Corn	0.0017**	NA	Krupke *et al.* 2012
Trifloxystrobin	NA	Corn	0.0017**	NA	Krupke *et al.* 2012
Unnamed Chem # 2	NA	Canola	<0.00125+	<0.00125+	Study 2-5
Thiamethoxam	0.75	Pumpkin	<0.0002+	<0.0002+	Dively and Kammel 2012
Imidacloprid	NA	Mustard	<0.010+	<0.010+	Choudhary and Sharma 2008
Clothianidin	1.0	Melon	ND	ND	Bocksch 2010
imidacloprid	0.33	Melon	ND	ND	Bocksch 2010

NA = not available ND= non-detectable
*Represents maximum
**Represents mean
+Chemical was not detected. Level of detection is set as upper bound.

Table 18 includes the proposed residue values expressed as a dose (μg a.i./bee). The dietary-based exposure value for adults is 0.29 μg a.i./kg for exposure through consumption of nectar and pollen. The dietary-based exposure value for larvae is 0.120 μg a.i./bee. The strengths of this approach are that it is empirically based and simple to use. At the Tier I screening level, the values in **Table 18** can be applied to all chemicals that are applied to seeds, with no need for adjustment based on application rate or chemical properties.

Table 18. Tier I exposure values used to estimate high end exposures of honey bees to pesticides applied via seed treatments.

Life Stage	Exposure Type	Dietary Dose (µg a.i./bee)
Adult	Diet (nectar + pollen)	0.29
Larvae	Diet (nectar + pollen)	0.12

3.4.1.1 Assumptions and Uncertainties

As indicated above, the proposed use of the EPPO screening-level exposure value of 1 mg a.i./kg for pollen and nectar of seed treated crops does not account for differences in application rate (*i.e.*, the mass of the pesticide applied to the seed). There is uncertainty in not accounting for the mass of the chemical applied since it is expected that the magnitude of the pesticide concentration in the plant will be influenced by the magnitude of the application; however the evaluation of this screen indicates that is protective for rates that are currently being used for seed treatments. The pesticide application rates of the available data used to evaluate the 1 mg a.i./kg screening value range 0.7-1.25 mg a.i./seed. Therefore, the 1 mg a.i./kg screening value is expected to apply to pesticide treatments that are at a similar rate. If application rates are much greater, pesticide concentrations in pollen and nectar may be higher than the empirical data provided in **Table 17**. However, even if pesticide concentrations of seed treatments exceed 1.25 mg a.i/seed, it seems unlikely that pesticide concentrations in nectar and pollen would exceed the 1 mg a.i./kg value since 1) the screening value is a factor of 28 greater than the highest residue measured in pollen and nectar, and 2) pesticide mass applied to seeds is not expected to be much greater than 1.25 mg a.i/seed since this value is already similar to the overall weight of the seed itself.

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In addition to the EPPO (2010) screening value, available uptake models were considered. No uptake models were identified where pesticide concentrations could be estimated in plants after seed treatments. As an alternative, the soil-plant uptake model developed by Briggs *et al.* 1982 and 1983, with modifications by Ryan *et al.* (1988) was considered as a potential method that may be used to estimate upper-bound exposures of bees to pesticides applied as seed treatments. In order to apply such a model, it is necessary to estimate the amount of pesticide present in the soil of the treatment site. For pesticide applications that are made via seed treatments, which are expressed on a mass of pesticide per seed (*i.e.*, mg a.i./seed) or mass of pesticide per mass of seed (*e.g.*, mg a.i./cwt seeds) basis, several assumptions would need to be made to determine the mass of pesticide available in a volume of soil. For example, it would be necessary to determine how deep the seed is planted, how many seeds are planted per area, and how much mass of pesticide is available for uptake. Given that these parameters are expected to vary by crop and even by region, there would be a great deal of uncertainty in assigning representative parameter values to represent these variables. Therefore, it was determined that the application of the soil to plant uptake model developed by Briggs *et al.* 1982 and 1983, with modifications by Ryan *et al.* 1988 would generate estimates of pesticide concentrations in nectar and pollen that have greater uncertainty when compared to the EU's screening value, which is based on empirical measures in plants following both soil and seed treatments.

3.5 Quantifying Pesticide Exposure from Tree Injections and Trunk Drenches

3.5.1 Method Description and Evaluation

No empirically based models were identified to estimate pesticide concentrations in pollen, nectar or leaves of trees injected or drenched with pesticides. For screening-level purposes, the proposed method is to estimate the concentration in the vegetative part of the treated tree by dividing the mass of the pesticide applied to the tree by the mass of tree vegetation represented primarily by leaves, but also by flowers. This approach assumes that the applied pesticide is homogenously distributed in the tree's leaves and flowers and is not present in other parts of the tree. This approach can be evaluated using data from two registrant submitted studies involving trunk drench applications of imidacloprid to citrus trees (Bryne *et al.* 2011) and Horse Chestnut trees (*Aesculus hippocastanum*) (Maus *et* al. 2004).

The highest measured concentration of total imidacloprid (parent and degradates) in citrus nectar, in Bryne *et al.* 2011, was 0.11 μg a.i./L, which is approximately 0.18 mg a.i./kg. In this study, imidacloprid was applied at a rate of 0.0016 lb a.i./tree (726 mg a.i./tree). The approach described above can be applied to these application scenarios using the weight of citrus leaves and the weight of hardwood tree leaves. For citrus trees, Alva *et al.* 2003 reported that 32 month old orange trees had 0.600-1.000 kg of leaves (dry weight) per tree. If it is assumed that leaves are represented by 80% water (see T-REX user's guide), this is equivalent to 3-5 kg wet weight of leaves per tree. If the mass of imidacloprid applied to the citrus trees included in the study conducted by Bryne *et al.* 2011 (*i.e.*, 726 mg a.i./tree) is divided by the mass of leaves per tree, the result is an estimated concentration of 242-145 mg a.i./kg. This value is three orders of magnitude above the reported concentration of imidacloprid in nectar (*i.e.*, 0.18 mg a.i./kg).

The highest measured concentration of total imidacloprid (parent and degradates) in chestnut tree leaves (Maus *et* al. 2004) was 4.64 mg a.i./kg. In this study, imidacloprid was applied at a rate of 0.06 g a.i. per centimeter of stem diameter. Applications for the four trees in the study ranged from 0.003 – 0.004 lb a.i./tree (1320 – 1760 mg a.i./tree, depending on the tree diameter). For chestnut trees, leaf biomass for hardwoods can be estimated using general hardwood leaf biomass equation from Sollins *et* al. 1973, as adapted by Jenkins *et* al. 2003. Using this equation, the equivalent wet weight of leaves per tree ranges from 31 – 50 kg. If the mass of imidacloprid applied to the chestnut trees included in the study conducted by Maus *et al.* 2004 (*i.e.*, 1320 – 1760 mg a.i./tree) is divided by the mass of leaves per tree, the result is an estimated concentration ranging from 38 – 47 mg a.i./kg. The measured value of imidacloprid in leaves is one order of magnitude higher than the highest measured concentration of total imidacloprid in leaves.

3.5.1.1 Assumptions and Uncertainties

One major uncertainty associated with this approach is the estimate of the vegetative mass of the tree, which can vary greatly based on species, age, time of the year and geography. In addition, in this approach, it is assumed that 100% of the active ingredient is taken up into the tree and moved into the leaves and flowers. It is unlikely that all of the pesticide mass applied to the tree actually enters the tree, or that all of this is moved solely to the leaves.

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3.6 Discussion of Potential Exposures through Other Routes besides Diet and Direct Spray

3.6.1 Contact with Dust

Another potentially important route of exposure for bees is contact with dusts that occurs from direct application of a pesticide. Bees could be exposed to pesticides in dust while in flight (contact exposure), or through flowers where dust has been deposited (contact or dietary exposure). Possible sources include dust generated through abraded seed coat dust during planting, disruption of treated soil through farming activity, and generation of contaminated dusts through the harvesting of treated plants. Of these potential sources of contaminated dust, only the exposure to dusts generated through abrasion of treated seed coat has been associated with incidents of honey bee mortality (Pistorius *et al.* 2009, Forster *et al.* 2009) and is the focus of considerable research (*e.g.*, Taparro *et al.* 2012, Krupke *et al.* 2012). EFSA 2012 as well as the recent SETAC Pellston workshop (Fischer and Moriarty 2012) also identified exposure to dust drift from sowing treated seed as a relevant exposure route for bees. However, methods of consistently quantifying exposures to dusts have not been well defined. The extent to which honey bees are exposed via contact with abraded seed coat dust is determined by many factors, including the physico-chemical properties of the seed coating, seed planting equipment, use of seed delivery agents (*e.g.*, talc and graphite), environmental conditions (wind speed, humidity), and hive location in relation to sowing. These factors influence the amount of dust formed, the pesticide concentration in the dust, and depending on dust particle size and weather, the extent to which the dust may move.

EPA, PMRA and CalDPR are not aware of any validated models to assess this exposure route. A field-scale model for dust emissions from planting of treated seed could potentially be developed using the AERMOD model (USEPA 2012*f*). Parameterization of this model is complicated because of the number and variety of variables that determine the importance of this exposure route and associated risks (*e.g.,* type of equipment, use of talc, seed coating material, climatological conditions, and particle size). An empirically based approach involving the available studies documenting incidents associated with dust exposures (Taparro *et al.* 2012, Krupke *et al.* 2012) could be used to derive parameters for AERMOD; however, given the extremely high concentrations of insecticides found in solid lubricants for seed

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planting equipment, it is unlikely that a screening model based on such source terms would be useful to discriminate among insecticides' risks to bees.

Relative to potential exposure through drift of abraded seed coat, the EPA and PMRA are currently investigating the mortality incidents that have occurred in the U.S. and Canada in 2012 to determine whether the exposures through dust can be associated with specific application and/or weather conditions. This work may be used to inform future assessment methods of this route of exposure. At this time, EPA and PMRA are working with pesticide registrants, seed treatment companies and seeding equipment manufacturers to better ensure the development and use of appropriate sticking agents, seeding equipment lubricating agents, and more effective venting/filtering options for such equipment to minimize the evolution of dust at the time of planting so that exposure to honey bees through contaminated dust is minimized.

3.6.2 Consumption of Contaminated Drinking Water

In order to understand the potential pesticide exposure to bees through consumption of water, it is important to be able to estimate pesticide concentrations in the different types of water bodies from which bees may collect water, as well as the amounts of water bees will collect from each source. Pesticide concentrations are expected to vary among potential sources of drinking water. An analysis discussed in detail in **Appendix 2** includes estimated pesticide concentrations in ponds, puddles and dew, which are all considered relevant sources of water for bees. In estimating the total pesticide exposure a single bee may get from drinking water, there are two major sources of uncertainty: 1) the major source(s) of water used by bees are unknown, and 2) the amount of water a bee will actually drink is unknown. **Appendix 2** includes an analysis of two potential drinking water intake rates for bees using two different methods, where one is based on behavioral observations of honey bees and the other is based on direct measurements of water flux rates of a similar species (i.e., the brown paper wasp). The analysis showed that the two methods generate results that differ by orders of magnitude, and concluded that the method based on water flux rates in the brown paper wasp is more reliable. Upper bound pesticide concentrations were estimated in various sources of water that bees potentially use for drinking water, including ponds (adjacent to treated fields), on-field puddles and dew (present on crops) In addition, potential exposures through contaminated guttation fluid are also discussed using available empirical measures of pesticide concentrations in this fluid. Guttation fluid has been discussed in the

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scientific literature in recent years as being a potential drinking water exposure route of concern due to its high (ppm level) concentrations of some pesticides following pesticide treatments.

Based on estimated pesticide concentrations in various sources of water potentially consumed by bees and a water flux rate from the brown paper wasp, the results of this analysis indicate that if bees consume the majority of their water from puddles or ponds, the exposures relative to dietary and direct spray are insignificant. If bees drink a substantial amount of water from guttation fluid or dew, conservative exposures may be similar to or even exceed pesticide exposures through the diet or direct spray. Because this indicates a potential concern for assessing exposures of honey bees to pesticides, potential exposures through drinking dew and guttation fluid were investigated further. This investigation concluded that pesticide exposures through dew and guttation fluid are not expected to be as significant when compared to diet because of two primary reasons. First, although the importance of dew and guttation fluid to bees as a source of drinking water is unknown, dew and guttation fluid are only expected to be present during a portion of the morning which would prevent bees from drinking a substantial amount of water from these sources. Second, for many worker bees, pesticide doses through consumption of dew and guttation fluid may be much less due to lower or non-existent drinking water consumption rates (because of higher amounts of water consumed through food). Therefore, pesticide exposure through drinking water is not included in the proposed Tier I exposure route for bees.

3.7 Tier II Exposure Assessment for Honey Bees

As discussed previously, the risk assessment process is intended to be iterative. At the Tier I level, exposure values used to estimate RQs are intended to be conservative; however, for chemicals which do not pass the Tier I level, increasingly refined estimates of exposure may be needed to provide a more realistic understanding of pesticide exposures to honey bees under actual use conditions. Therefore, the purpose of the Tier II exposure assessment is to obtain pesticide specific, empirically-based exposure data that potentially represent doses received by bees. Like the Tier I exposure method, potential exposures should be assessed through quantification of doses through direct spray or diet. Specifically, studies should be designed to quantify pesticide residues in pollen, nectar and on bees located on treated fields.

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The strength of basing the Tier II exposure approach on empirical data from field studies is that some of the uncertainties associated with the Tier I exposure method are reduced or eliminated. For instance, the assumption incorporated into the Tier I exposure assessment for foliar applications that pesticide concentrations on grass are equivalent to nectar and pollen is not necessary if pesticide concentrations are quantified directly in nectar and pollen. Also, the Tier I use of upper-bound exposure values based on a large set of chemicals is not necessary if pesticide-specific residue levels can be established under field conditions that are representative of the pesticide's intended use patterns. One remaining uncertainty that is associated with chemical-specific measures of pesticides in nectar and pollen is that the actual pesticide doses received by bees through diet are still unknown due to the need to convert measured concentrations in pollen and nectar to dose using food consumption data (*i.e.,* Rortais *et al.* 2005).

This section describes the basic objectives and design elements of two types of field studies that can be used to quantify pesticide concentrations in matrices that are relevant to bees. The first study is a targeted field study where pesticide residues are quantified in pollen and nectar collected from treated crops. This study could be specifically designed with the sole intent of quantifying pesticide residues in matrices relevant to honey bees, or it could be incorporated into a study design of an existing study required for pesticides, such as a cropped TFD study (OCSPP guideline 835.6100; USEPA 2008). The second study is referred to as a tunnel study because the treated crop is kept under an enclosure (referred to as a tunnel) along with a nucleus hive of bees. Pesticide concentrations can be quantified from the stomach contents, pollen sacs, pollen traps attached to the hive and comb of the bees and their hive which is maintained within the tunnel. Depending on the plant species, both pollen and nectar may be readily available for direct collection by researchers. In these cases, the targeted field study may be preferred for generating Tier II exposure data. In cases where it is logistically difficult for researchers to collect pollen and nectar samples by hand, honey bees may serve as a reasonable means to collect samples. In those cases, tunnel studies may be preferred.

The sampling scheme is an important consideration in the design of the study. Sampling should target the maximum concentrations found in pollen and nectar; therefore, these studies should sample pollen and/or nectar at different time points during bloom to evaluate the presence and the decline of the compound over time. The sampling should also be specific to the target crop and application method. For foliar applications, studies should be designed such that pesticide concentrations are quantified on

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the day of application (presumably to quantify the highest pesticide residues) and during subsequent days that are selected to represent the concentrations on bees and relevant plant samples over time. These sampling dates should be selected based on consideration of two factors: dissipation of the chemical through degradation and transport away from the plant (*e.g.,* via volatilization) as well as systemic transport through the plant to pollen and nectar. For soil and seed treatments, pesticide concentrations should also be quantified in nectar, pollen, at the time of flowering. In these studies, pesticide degradates of concern should also be quantified. These degradates can be identified using available registrant-submitted environmental fate and plant metabolism studies (Guideline 860.1300; USEPA 1996*b*).

There are several sources of variation related to the anticipated use of the pesticide that should be considered when designing studies for the Tier II exposure assessment, including application methods, crops and location. Application methods employed for a chemical can vary. Because different application methods may result in different levels of exposure, application practices that are expected to generate high-end exposure concentrations for the pesticide should be selected. In determining which crops to include in field studies, a focus should be placed on registered uses for crops that are highly attractive to pollinators and the crops that have the highest application rates as well as the potential to be visited by pollinators. For systemic pesticides in particular, different plant species may have differences in uptake, distribution, and metabolism of the test substance. In addition, different plants may have different matrices that represent food sources for bees. For example, corn only produces pollen that bees will collect as a food source; canola produces both nectar and pollen (in flowers). Some plants, such as cotton also produce extra-floral nectaries which may be present when flowers are not. Different field locations have different climates, soils, and therefore growing conditions at a spatial scale. The locations of the studies should be selected so that they represent the anticipated use sites of the chemical. If possible, like with MOR studies, pollinator-specific studies could be conducted in multiple regions that are identified to be of concern. As a result of these considerations, it may be necessary to conduct targeted studies using multiple application methods, crops and locations in order to quantify potential pesticide exposures to bees under conditions that are representative of the anticipated use of the chemical. Because field residue data may exhibit extreme spatially and temporal variability, it is necessary to collect a sufficient number of data from different studies in order to obtain a sample that represents the potential environmental exposures. A sufficient number of studies should be collected to allow the risk assessor to have an understanding of the central tendency and upper-

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bound exposure values. The upper-bound exposure values (*e.g.*, 90th or 95th percentiles) from Tier II exposure values may be used for risk assessment purposes.

The field and tunnel (semi-field) residue studies can provide important information regarding the levels of contamination potentially encountered by bees in the field; however, there are a number of uncertainties associated with these studies. One major uncertainty is that the levels of residues inside the hive may be different than those first encountered by bees in the field. Bee bread and honey that form the basis of the hive diet undergo processing by bees with various enzymes and microorganisms prior to feeding most of the castes of hive bees. Only foragers will consume nectar directly as a major food source to meet their metabolic requirements during foraging. Consequently, measurements of residue levels in pollen and nectar collected directly from the flower or from returning bees may be different from the exposure to individuals within the hive. Furthermore, bees will typically collect a variety of food sources that would dilute any contamination from the test crop, unless other sources of contamination are also brought into the hive. Therefore, pesticide concentrations in freshly collected nectar and pollen could differ from potential residue levels in honey and bee bread, respectively.

In addition, one notable limitation to the Tier II exposure assessment method is that it will require a substantial amount of time and resources to complete. In the future, it is possible that the data acquired from targeted monitoring studies may be used to establish patterns of exposure based on application methods, crops and location. These patterns could then be used to develop a refined method where the data could be extrapolated to other chemicals. In addition, some field and tunnel residue studies that are currently being conducted also involve the collection of data on residues in leaves and blossoms. These data could potentially provide a more complete dataset from which to evaluate the ability of blossom or leaf residues to provide a conservative measure of residues in pollen and nectar.

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4 Characterization of Ecological Effects

At this time, the first tier of testing in North America consists of an acute contact toxicity test, *i.e.*, OCSPP Guideline 850.3020 (USEPA 2012*a*) and OECD Guideline 214 (OECD 1998*a*) on adult honey bees that provides a median Lethal Dose (LD_{50}), *i.e*, the lethal dose that causes the death of 50% of the exposed organisms. In addition to a contact toxicity test, an acute oral toxicity test is also required in Canada that provides information on the oral LD_{50} from a single dose of the test compound, along with any sublethal effects (OECD Guideline 213; OECD 1998*b*). Depending on the outcome of these toxicity tests, pesticides are classified as practically non-toxic (LD_{50}>11 μg a.i./bee), moderately toxic (2<LD_{50}<10.99 μg a.i./bee), or highly toxic (LD_{50}<2 μg a.i./bee) to bees on an acute exposure basis. If the acute contact LD_{50} is less than 11 μg a.i./bee, additional testing may be required in the form of a foliar residue study (OCSPP Guideline 850.3030; USEPA 2012*b*) to determine the duration over which field-weathered foliar residues remain toxic to honey bees. On a case-by-case basis, EPA and PMRA may also require additional higher-tiered studies such as hive studies and semi-field and field pollinator studies (*i.e.*, OCSPP Guideline 850.3040 (USEPA 2012*c*), OECD Guidance Document 75 (OECD 2007), and the EPPO Standards PP1/170 (EPPO 2010) if the data from toxicity studies indicate potential chronic effects or adverse effects on colonies. The ecological risk assessment method proposed in this white paper would expand the current data requirements substantially.

Expansion of Tier I testing for pollinators was proposed in a USDA-led workshop, as well as in two recent publications. In 2009, EPA hosted a USDA-led workshop to discuss the development of new toxicity testing protocols for determining the potential effects of pesticides on bees. This workshop included representatives of CalDPR, PMRA, the French Ministry of Agriculture, and university researchers and focused on laboratory, semi-field, and field toxicity testing protocols for honey bees and other non-target invertebrate pollinators. Proceedings from the workshop (USDA 2010) indicated the need for acute oral toxicity testing, effects testing with non-adult bees, better standardization of sublethal effects to be included in toxicity tests, and the development of standardized methods for higher tiered studies using colonies under more realistic field conditions. In addition, the summary of a 2011 SETAC Pellston Workshop (Fischer and Moriarty 2011) recommended inclusion of both oral and contact acute toxicity testing on adult bees in Tier I, as well as larval testing where larval exposure is possible. Another recommendation was to include chronic toxicity testing with adult bees "as soon as test methodologies can be verified." EFSA 2012 made similar recommendations and also noted the importance of making

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precise observations of sublethal effects that might occur during current testing, ultimately recommending that tests to evaluate sublethal effects should be considered for future Tier I testing.

4.1 Tier I Effects Assessment

The purpose of the ecological effects assessment is to describe the effects elicited by a stressor (*i.e.*, pesticide active ingredient). This involves linking observed effects to the assessment endpoints and evaluating whether a cause-effect relationship exists (USEPA 1998). During the Tier 1 stage of the risk assessment, effects data are evaluated to determine whether they are consistent with assessment endpoints and to confirm that the conditions under which they occur are consistent with the conceptual model depicted in the problem formulation. This section of the white paper discusses the ecological effect studies which are proposed for the screening-level (Tier 1) risk assessment. While many of the studies discussed in this section are routinely required by the EPA and PMRA, other studies are relatively new and in some cases, the testing procedures for these newer studies (*e.g.*, acute larval and chronic toxicity tests) are still in development. As discussed in the exposure characterization (Section 3), the major routes of exposure are expected to be contact and dietary (*i.e.,* oral). Therefore, at the Tier I level, toxicity tests are intended to assess the effects of a chemical when bees are exposed in this manner.

4.1.1 Acute Toxicity

The proposed Tier I method for characterizing effects of pesticides on bees following acute exposures is based on three types of studies. These include: acute contact toxicity for adult worker bees, acute oral toxicity for adult worker bees and acute oral toxicity for worker larvae. The most sensitive LD_{50} values representing each of these exposure routes and life stages are intended to be used in combination with the appropriate estimated exposure value to derive RQs for the Tier I risk assessment. In cases where a pesticide is not applied via foliar spray, the acute contact toxicity data may not be necessary for assessing the risks of a chemical because for soil applications, seed treatments and tree trunk applications, dietary exposure is expected to be the predominant route.

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4.1.1.1 Contact Exposure to Adult Worker Bees

As noted previously, the current screening-level toxicity study for bees that is required in the U.S. is an acute contact toxicity test. According to 40CFR158 data requirements for pesticides (CFR 40 2012), these data are required when the pesticide is applied to crops which are known to be attractive to bees, or has other outdoor use patterns (such as forestry or residential) which may result in bee exposure. The test design and method for deriving acute contact toxicity data for adult worker bees are well established, with guidelines available through EPA Guideline 850.3020 (USEPA 2012*a*) and OECD Test Number 214 (OECD 1998*a*). The purpose of this study is to quantify the dose-response relationship, with the response measured by bee mortality following contact exposures to a pesticide. The standard acute contact toxicity test methodology involves application of the technical grade active ingredient in a solvent (or in a formulated product), applied by microapplicator to the thorax of adult test bees. Adult worker bees are exposed to five doses in a geometric series of the test substance. A minimum of three replicate test groups are used for each test concentration, with each replicate containing ten bees. Each test includes a control group (treated with the solvent). In addition, a positive control involving exposures of bees to a toxic standard (*e.g.*, dimethoate) is typically included in the test. The test duration is 48 hrs; however, if the mortality rate continues to increase between 24 and 48 hrs, the test may be extended to 96 hrs. The primary measurement endpoint is the 48-hr LD_{50} (expressed in units of µg a.i./bee). Sublethal effects are also reported in acute contact studies; therefore, depending on the nature of those effects and whether a dose-response relationship is observed, the study may yield a median effect dose (ED_{50}). Because the focus of this test is on quantifying the mortality, the dose levels of these tests are not designed to quantify an ED_{50} with confidence (EFSA 2012).

Based on the median lethal contact dose for 50% of the bees tested (*i.e.*, contact LD_{50}) from this study, the pesticide is classified as practically non-toxic ($LD_{50} \geq 11$ µg/bee), moderately toxic ($10.9 > LD_{50} \geq 2$ µg/bee), or highly toxic (<2 µg/bee). The proposed Tier I risk assessment method involves using the 48 or 96 hour LD_{50} generated from an acute contact laboratory study to derive RQs representing risks to forager bees exposed to pesticides from contact (following foliar spray applications). Unless the pesticide is determined to be practically non-toxic, EPA would then typically require a study on the toxicity of residues on foliage to honey bees (OSCPP 850.3030; USEPA 2012*b*).

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The current OSCPP guideline study 850.3030 (USEPA 2012*b*) evaluates the toxicity of residues on foliage. In this study, a formulated product of a chemical is applied to a bee attractive plant (*e.g.*, clover or alfalfa) at the maximum application rate with the minimum application interval. The crop is then harvested in a manner that provides different age residues (*e.g.,* 0, 24, 48, 72 and 96 hours after application). The harvested foliage is brought back to the laboratory where it is placed into cages. Adult bees are placed in each cage with the foliage and allowed to come into contact with the foliage. Food and water are provided *ad libitum*. The bees are monitored for a period of time until mortality declines to below 25%. The measurement endpoint derived from this study is the RT_{25}, which is defined as the time needed to reduce the "residual toxicity" of the test substance, as measured by mortality, to 25%. The time period determined by this toxicity value is considered to be the time that the test substance is expected to remain toxic to bees in the field from the residual exposure of the test substance on vegetation at an expressed rate of application (typically expressed in terms of lb a.i./A). Although the results of this study would be useful in the characterization of effects and risks of a pesticide to honey bees (*e.g.,* through a discussion of how long a pesticide may be expected to present a risk to foraging honey bees), the proposed Tier I risk assessment method does not include using the data generated from this type of study to derive RQs.

4.1.1.2 Oral (Dietary) Exposure to Adult Worker Bees

Although not currently required by EPA, PMRA and the EU routinely require acute oral toxicity data for adult worker bees. The acute oral toxicity study is a laboratory test method, designed to assess the mortality of young adult worker bees following a single oral dose of a pesticide. A standard OECD guideline exists for this study, *i.e.*, OECD Guideline 213 (OECD 1998*b*). According to the OECD guideline, adult worker honey bees are exposed to five doses in a geometric series of the test substance dispersed in sucrose solution. A minimum of three replicate test groups, each with ten bees, is then dosed with each test concentration. As with the acute contact study, a toxic standard (usually dimethoate) is typically included in the test series to serve as a reference (positive) control. The bees are then fed the same diet, free of the test substance. Similar to the acute contact toxicity test required by EPA, the study provides a 48-hr LD_{50} value and can be extended to include a 96-hr LD_{50} value if mortality is demonstrated to increase between 24 and 48 hours. In addition, the study should also generate some observations of sublethal effects that could potentially be used to develop an ED_{50} value. The proposed

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Tier I risk assessment method involves using the 48 or 96 hour LD_{50} generated from an acute oral laboratory study to derive RQs representing risks to adult bees exposed to pesticides through the diet (following foliar spray applications, soil application, seed treatments and tree trunk treatments of pesticides).

4.1.1.3 Oral Exposure to Larval Worker Bees

Unlike with the acute contact and oral toxicity studies for adult worker bees, established regulatory test guidelines for assessing larval toxicity do not exist. Given that larvae may be exposed to pesticides through the diet, the lack of understanding of the toxicity of a chemical on larvae is considered to be a significant data gap. Therefore, efforts are underway to identify critical design elements of an acute oral larval toxicity study that may be used to understand the toxicity of a chemical to this life stage of worker bees. Several larval toxicity studies have been published in the literature that may be useful in establishing an acceptable acute oral larval toxicity studies (*e.g.,* Aupinel *et al*. 2007 and OECD 2007).

One option is an *in vitro* study designed to measure the acute toxicity of a technical grade active ingredient directly applied to honey bee larvae. Assays where individual bees have been removed from their colonies can be thought of as *in vitro* studies since the bees are outside of the 'superorganism'. Aupinel *et al*. 2007 proposed an *in vitro* method for assessing the effect of pesticides to honey bee brood which allows the quantification of doses ingested by larvae and the assessment of larval and pupal mortality as well as sublethal effects. In this method, bee larvae (1st instar) are transferred into 48-well plates and fed royal jelly containing known quantities of the test chemical over the 48-hr study period. The test provides LD_{50} values and depending on the extent of replication can provide NOAEC values as well. The methodology was subsequently ring tested and a review (Aupinel *et al.* 2009) indicated that additional adjustment in the method may be needed to address potential sources of variability (*e.g.*, colony origin of the brood, season and larval heterogeneity at grafting).

Alternatively, the screening-level assessment of larval toxicity could be based on a honey bee brood study using in-hive exposure of brood fed by adults. The methodology for this *in vivo* study (*i.e.*, within the "superorganism") was first described by Oomen *et al*. 1992 and later developed into an OECD Guidance document (OECD 2007) based on Schur *et al*. 2003 using whole colonies. These methods however, are not considered to be laboratory-based studies. Rather they are considered semi-field

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studies since they entail using honey bee colonies and are conducted in a tunnel even though the original study method by Oomen *et al*. 1992 involved bee colonies which were not confined to a tunnel.

The OECD guidance (OECD 2007) suggests that an *in vitro* laboratory-based study may be an appropriate first step in determining whether the colony study is warranted. Consistent with this recommendation, for assessing the acute toxicity of a pesticide to larvae that are exposed through the diet, the proposed Tier I risk assessment method is to use an LD_{50} value generated from an *in vitro* test with honey bee larvae, similar to the method used by Aupinel *et al*. 2009.

One notable uncertainty associate with the Aupinel *et al*. 2009 method, as well as existing chronic toxicity tests with larvae, is that they rely on a synthetic food matrix to which the test chemical is added. The uncertainty surrounds the extent to which this food is reflective of the brood food and/or royal jelly that developing larvae are typically fed. The manipulation of bee larvae outside of the colony environment also introduces uncertainties regarding how such manipulations affect bees in terms of the trophallaxis, *i.e*., sharing of food, on which they naturally depend. This is particularly true for bee larvae which consume a highly processed food as opposed to the matrix provided in some of the currently available study methods.

4.1.1.4 Other Acute Toxicity Data

Additional data are often available that may be useful in characterizing the effects of a pesticide on bees. The scientific literature often includes toxicity data involving bees. In cases where more sensitive acute toxicity data for honey bees are available in scientifically valid studies from the scientific literature, these values may be used to derive RQs (instead of less sensitive endpoints from registrant-submitted studies). In addition, there are cases where data that are not used to derive RQs are considered in the effects characterization. For example, data are often available from registrant-submitted acute toxicity studies involving other non-target arthropods (*e.g.,* green lacewing, non-parasitoid wasps). Also, reports of honey bee mortality events (beekill incident reports) following pesticide exposures may be useful in evaluating the available laboratory toxicity data.

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4.1.2 Chronic Toxicity

Chronic laboratory-based toxicity testing has not been routinely required on individual bees in the US; information on the potential chronic toxicity of pesticides to bees has historically been discerned from whole colony studies conducted under semi-field or full field conditions. The USDA Technical Working Group report (USDA 2009) noted that chronic toxicity tests with individual adult bees have been conducted to support registrations in the EU. These tests consist of collecting newly emerged (juvenile) bees from comb and placing 50 of these bees per cage. A sufficient number of replicates are run along with suitable negative and reference controls to conduct hypothesis testing to establish a NOAEL and LOAEL. The bees are fed a sucrose solution; however, no protein source (pollen) is provided. Exposure is through spikes made to the sucrose solution; cages of bees are provided 10 mL of test solution every other day. Bees are observed for a total of 10 days and mortality (chronic survival) is the primary measurement endpoint. In discussions at the USDA workshop, participants suggested that the exposure source to the bees could vary depending on the primary route of exposure expected for the chemical, *i.e.*, either through nectar (sucrose) or through pollen. However, exposure through pollen can present logistical challenges as bees may inadvertently walk through the treated pollen where exposure may be extended to contact rather than simply through oral ingestion. Although the primary endpoint measured has been mortality, additional measurement endpoints may include adult bee longevity, body weight, and immune response.

The proposed Tier I risk assessment method for bees includes quantification of the toxicity of larvae and adult worker bees following chronic exposures to pesticides; however, no formal guidelines have been developed to date for conducting chronic toxicity tests with either adult or larval bees. Therefore at this time, evaluation of the potential chronic effects of pesticides on individual bees is not possible; however, efforts are underway to identify design elements of a chronic larval and adult toxicity studies that may be used to understand the toxicity of a chemical to worker bees. Critical questions related to this process include:

- What is an appropriate duration to represent a chronic exposure to larval and adult worker bees?
- What is an achievable duration of exposure given husbandry limitations of honey bees?

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- What endpoints are meaningful for understanding the potential impacts of a pesticide on the assessment endpoints (*i.e.*, maintaining honey bee population size, stability of managed bees, quality and quantity of hive products, species richness and abundance)?
- How should those endpoints be quantified (*e.g.*, EC_{10}, EC_{20}, EC_x, NOAEC, LOAEC)?

These questions are explored in the following sections that focus on the duration of chronic effects tests and the endpoints measured in those tests. Also discussed below are some uncertainties in conducting laboratory toxicity tests with bees that may be necessary to consider when designing chronic toxicity studies.

4.1.2.1 Duration of Chronic Tests

The term "chronic" is intended to represent repeated exposures that occur over an extended period of time, generally representing a substantial portion of an organism's life. Because different organisms have different life expectancies, the actual duration of a chronic exposure may differ by organism. For example, because a summer worker bee lives for a period of several weeks, a chronic exposure for a worker bee would be measured by weeks. Because a queen bee may live for years, a chronic exposure for the queen may be measured by months or years. Therefore, in establishing a chronic test for worker bees, it is necessary to select appropriate test durations so that the end result represents an exposure that is chronic.

As indicated in the problem formulation, the proposed Tier I method for assessing risks of pesticides to bees involves establishing separate endpoints for worker larvae and adults. The duration of the worker larvae life stage is approximately 7 days (Wilson 1987), with the first 5 days occurring while the larvae are uncapped and fed by nurse bees. Chronic larvae toxicity tests have been carried out where bees were observed for 7 or more days following exposures. Efforts to extend larval toxicity tests beyond 7 days to include the pupation stage have frequently resulted in increased mortality (>10%) of the developing bees. Therefore, the 7-day exposure period appears to be an appropriate duration of exposure as it covers a large proportion of the larval life stage and extension into the pupal life stage may not be possible due to husbandry concerns. The proposed Tier I method for deriving chronic RQs for larvae worker bees would be based on toxicity tests with 7-day exposure periods.

The lifespan of an adult, summer worker bee is several weeks. Some laboratory tests have been developed where the toxicity of adult bees has been examined over a 10-day exposure period (EFSA 2012). This also appears to be an appropriate duration of exposure to represent chronic effects to adult worker bees as it represents 25-50% of the adult lifespan. Therefore, the proposed Tier I method for

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deriving chronic RQs for adult worker bees would be based on toxicity tests with 10-day exposure periods.

4.1.2.1.1 Mortality

As discussed in the problem formulation, the assessment endpoints for the ecological risk assessment of bees involve maintaining honey bee population size, stability of managed bees, quality and quantity of hive products, species richness and abundance. It is important to identify specific endpoints which will be measured in toxicity tests. These measurement endpoints must have clear linkages to assessment endpoints if they are to be used quantitatively (*i.e.*, to calculate RQs) in risk assessment. As indicated in **Table 1**, at the individual bee level (which is the focus of the Tier I assessment), measurement endpoints relevant to these assessment endpoints include: individual survival, adult bee longevity, brood size, brood success, and queen fecundity. Chronic toxicity tests with larvae and adults can be used to quantify effects of pesticides on all of these endpoints, with the exception of queen fecundity (which would require an egg laying study involving the queen). Therefore, the focus of the chronic toxicity tests with larvae and adults is on mortality that may occur during the tests. Potential impacts of a pesticide on brood size and success can be assessed by determining whether there is decrease in the number of brood (*i.e.*, larvae) following a chronic exposure of larvae to that pesticide. Potential impacts of a pesticide on adult survival and longevity can be assessed by determining the mortality and the decrease in the life spans of adult bees following chronic exposures to the pesticide. The notable limitation to the proposed chronic toxicity endpoints is that they do not include measures of queen fecundity.

In order to derive a RQ, the measurement endpoint must be quantified. For chronic toxicity studies, endpoints are traditionally represented by the No Observed Adverse Effects Concentration or Level (NOAEC or NOAEL). These values are calculated from hypothesis-based tests where the Lowest Observed Adverse Concentration or Level (LOAEC or LOAEL), which is determined from statistical or biological significance, is then used to establish the NOAEC or NOAEL. The NOAEC[L] is assumed to represent a lower-bound dose where no significant mortality is expected to occur in larvae or adults. The proposed method for establishing RQs for the Tier 1 assessment is based on NOAEL values from 7-d larval and 10-d adult studies, where the LOAELs are based on mortality or longevity (of adults).

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As an alternative, regression-based approaches have been proposed for quantifying measurement endpoints. Use of a regression-based endpoint would require determining what level of effect is relevant for a chronic exposure (*i.e.,* the percent decrease must be established). Since the Level of Concern for acute exposure has been established based on 10% mortality to foragers, a comparable endpoint for the chronic test would be an LD_{10}. In acute toxicity tests, the endpoint is the median lethal dose (LD_{50}), which is the most confident estimate of mortality generated from an acute toxicity study given that confidence intervals around the regression line (mean) are minimized at its center-most point, *i.e.*, the LD_{50}. Predictions of LD_x values that are on the low or high ends of the dose-response curve (*e.g.,* LD_{10} and LD_{90}, respectively), become less confident. This can be attributed to the selected test concentrations of the acute toxicity test, which are based on the goal of quantifying the LD_{50}. If the desired endpoint for a chronic study is an LD_x value, it would be necessary to select test doses that would be sufficient to confidently estimate that LD_x value.

4.1.2.1.2 Sublethal Effects

Although the proposed measurement endpoints for chronic exposures are based on mortality, there are an increasing number of sublethal endpoints reported in *Apis* and non-*Apis* toxicity studies which may be conceptually relevant to the proposed assessment endpoints. Sublethal effects have been defined as effects which do not directly cause the death of an individual organism (Thompson and Maus 2007); however, these same authors differentiate effects on reproduction (*e.g.*, death of larvae, failure of pupation or malformations during pre-imaginal development) as brood effects rather than sublethal effects. Examples of reported sublethal endpoints include impairments to movement and communication, foraging and homing behavior, recruitment of fellow foragers, health of the queen, detoxification pathways, immunodeficiency, and colony maintenance activity. Additional examples of sublethal effects reported in the open literature include data from proboscis extension response (PER) tests and flight tunnel studies. Proboscis extension response tests serve to show an insect's learning ability through olfactory conditioning, *i.e.,* a bee will learn to extend its proboscis when a sugar stimulus is touched to its antennae but will not when given a repellant stimulus. When a bee is unable to learn this behavior, this response may reflect an impairment of the bee's ability to forage and provide food for itself and the hive. Additionally, flight tunnel studies have been used to assess the homing ability of the bee, as well as a bee's ability to communicate a food source to other bees through the "waggle dance." EFSA's risk assessment of plant protection products on bees (EFSA, 2012) identified sublethal endpoints as being inadequately covered by existing test designs. Desneux *et al.* 2007 identified physiological and

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behavioral symptoms that can be observed and reported in laboratory studies. EFSA recommended that neurotoxicity symptoms (*e.g.*, movement and coordination), cleaning behavior, olfactory conditioning (learning and memory), enzymatic functions, and development of the hypopharyngeal gland tissues (responsible for producing brood/royal jelly) are important indicators of bee health and vitality and must be accounted for in the risk assessment process.

Before using these endpoints to derive RQs, the relevancy of these sublethal measurement endpoints for evaluating assessment endpoints needs to be established through quantitative links to colony strength and survival. Although such endpoints may not be used quantitatively to estimate potential effects on assessment endpoints, they could potentially be used qualitatively to characterize potential effects which may not have clearly established relationships to colony-level effects and for explaining mechanisms by which colony-level effects might occur. The SETAC Pellston workshop (Fischer and Moriarty 2011) as well as the EFSA opinion (EFSA 2012) both identified sublethal effects of pesticides on adult and larval bees as an important priority area for future research, especially in terms of developing tiered species-specific tests. Future work involving sublethal effects should also investigate their quantitative relationships with the assessment endpoints for risk assessment (**Table 1**).

Key science issues that must be addressed when considering sublethal effects include whether the length of a given study is sufficient to determine if a sublethal effect will lead to a survival, growth, and/or reproductive effect(s). Other factors to consider include the extent to which sublethal effects, observed in the laboratory and field, occur at environmentally relevant concentrations. While these are important areas for further evaluation, the scope of this white paper is intended to focus on the basic risk assessment process and underlying studies for determining whether exposure to a single chemical may result in adverse effects to the whole colony under actual use conditions. If there is sufficient information regarding a sublethal effect that provide evidence of a dose response and plausible linkages can be developed between the measurement endpoint and agency assessment endpoints, then such effects may have both qualitative and quantitative utility in a risk assessment. Where such information is not available, measurement endpoints may only have utility in qualitatively describing potential effects which have been associated with the pesticide. With respect to mixtures, while multiple stressors and the interactive effects of pesticides and/or other environmental stressors are important issues, they will not be examined at this time.

Sublethal effects measured in studies can span a broad range of biological levels of organization including altered biochemical pathways, genetic aberrations, changes in gut microflora, and behavioral

effects on the whole organism. However, there is an increasing need to understand how measurement endpoints at one level of biological organization relate to specific molecular initiating events (modes of action) and are part of a sequence of key events, which ultimately lead to an adverse outcome. Although adverse effects on survival, growth and reproduction in the intact animal may be more readily used in risk assessment, there is currently limited information available to quantitatively link many of the sublethal measurement endpoints with these apical endpoints.

Developing plausible hypotheses for potential mechanisms of action, which include key events and adverse outcomes, is increasingly important to understand and interpret the range of effects that may result across multiple levels of biological organization. These uncertainties underscore the need to delineate AOPs to better enable the extrapolation of measurement endpoints from one level of biological organization to another. Although it may not be necessary to completely delineate AOP(s), depending on the endpoint, there may be insufficient information available even on partially complete AOPs to enable risk assessors to reliably extrapolate the results to apical assessment endpoints on which the EPA, PMRA and CalDPR regulate. The need to identify a plausible modes of action is consistent with global efforts to identify and characterize molecular initiating events and other key events at various levels of biological organization that ultimately result in an adverse effect on the whole organism/population/community.

In order to link behavioral and physiological effects to survival, growth, and reproduction, there are threshold and temporal considerations. Threshold considerations include qualitative and quantitative measurements, such as the percent reduction in a particular measurement endpoint that will affect the vitality of an individual bee and ultimately that of the colony. Temporal considerations include transient effects, which must take into consideration the functions of different castes and life spans of bees and whether such transient effects are ultimately compensated for by the colony.

Although there is uncertainty in how sublethal measurement endpoints may be related to assessment endpoints, the EPA Overview Document (USEPA 2004) indicates that sublethal effect data may be considered in the assessment. This option is exercised on a case-by-case basis and with careful consideration of the nature of the sublethal effect measured and the extent and quality of available data to support establishment of a plausible relationship between the measure of effect (sublethal endpoint) and the assessment endpoints. This option includes a determination of whether there are clear, reasonable, and plausible links between the sublethal effect and survival or reproductive capacity of organisms in the field in accordance with the screening assessment endpoints of survival and

reproduction capacity. Although a number of statistically significant sublethal effects may be identified in a study between treated and untreated test organisms, these endpoints may have limited utility in a risk assessment unless they can be quantitatively linked to adverse effects for the whole organism, *i.e.*, colony, or population compared with an unaffected one (Thompson and Maus 2007). In the context of sublethal effects, there are no generally accepted thresholds of these effects, in terms of magnitude (EC_x versus NOAEC) for risk assessment. Thompson and Maus 2007 have discussed the need for validated assays for measuring such effects to achieve greater consistency and reliability in the results; however, at this time appropriate validation criteria have not been identified nor have appropriate reference standards for eliciting such effects been reported.

4.2 Tier II Effects Assessment

At a screening level (*i.e.*, Tier 1), effects data may primarily be based on laboratory studies while exposure estimates may be based on models and/or conservative default values. These studies are evaluated using relatively conservative assumptions to provide high-end estimates of potential risk such that if a chemical passes the screen, the presumption of low risk to non-target organisms is considered protective. As discussed in the section on problem formulation and which will be discussed in greater detail in the risk characterization section to follow, estimated exposure concentrations (EECs) are compared to acute toxicity endpoints (*i.e.*, LD_{50} values) from laboratory studies with individual bees. For those chemicals which fail to pass the screen (*i.e.*, the RQ value>LOC), additional testing/refinement may be required. As part of the tiered process, higher tier studies are intended to reflect increasing levels of realism, albeit from a less controlled testing environment, in terms of how organisms may be exposed and the nature of the effects which result from such exposure. This section discusses existing tests which have been developed and which could be used to refine the screening-level assessment.

As part of the tiered testing process for evaluating potential effects on bees, higher-tier refinements shift the focus from exposure and effects on individual bees to that of the intact colony. As noted in the preceding section, the transition to higher-tier toxicity tests may occur in advance of a formal risk assessment and may be predicated on the toxicity thresholds that were discussed earlier, *i.e.*, 40CFR158 testing requirements. As indicated in the 40CFR158, higher-tier testing of pollinators is currently triggered when specific criteria are met. For pesticides with an acute LD_{50}<11 μg a.i./bee and with extended residual toxicity (*e.g.*, RT_{25}>8 hrs) and/or data indicating the potential for adverse effects at the colony level, higher-tier (Tier III) field pollinator testing may be required. The decision to require

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additional toxicity testing is based on risk management needs and is typically made in the context of a risk assessment. However, the regulated community may elect to proceed with completing such studies based on their understanding of data requirements specified in the 40CFR158.

Unlike the screening-level studies, Tier II studies with bees are not typically conducted in the laboratory but rather focus on relatively controlled field studies (referred to as semi-field") where the movement of the bees to and from the colony is restricted through the use of enclosures containing treated crops and/or pesticide exposure is facilitated by feeding bees a pesticide-spiked diet. Compared to laboratory studies where exposure is to individual organisms and typically through a single route (*i.e.*, contact or oral), semi-field studies can provide clearer lines of evidence for linking multiple routes of exposure to adverse ecological effects. Semi-field studies are also useful for determining the extent to which effects on individual bees identified in Tier I laboratory studies are expressed at the colony level. Although the current EPA data requirements for pesticides identified in the 40CFR158 do not specifically include semi-field studies, the OCSPP Guideline 850.3040 on field testing for pollinators is general and would accommodate semi-field studies. Semi-field studies represent a reasonable and cost-effective means of addressing uncertainties and/or minimally refining the concerns regarding specific risks to bees in advance or in lieu of full-field testing.

The social nature of honey bees requires extensive interaction (*e.g.*, trophallaxis) and communication among various castes of bees for successful development and propagation of colonies. As a result, numerous linkages exist between pesticide exposure and effects on individual bees and their collective impact on honey bee colony health which are not readily assessed in the laboratory. Additionally, the Tier II semi-field studies provide a means of assessing both exposure and effects that are less conservative and more realistic than the methods used in laboratory testing. For example, the Tier 1 laboratory larval toxicity studies typically feed larvae with spiked sucrose solutions; however, early in their development, bee larvae do not typically consume pollen or nectar directly but rather consume brood food from the hypopharyngeal and mandibular glands of nurse bees. Thus, Tier II studies may be conducted if concerns remain from Tier I levels of the risk assessment and further information is needed for a more refined estimate of potential risk to honey bees. An example of such a refinement is the transition from *in vitro* testing of bee larvae proposed by Aupinel *et al*. 2007 to the *in vivo* colony-level brood study described in the OECD Guidance document (OECD 2007).

The design of the Tier II semi-field study is based upon the specific issues raised at the initial tiers of risk assessment. Consequently, the endpoints measured in the Tier II test should attempt to focus on those specific measureable endpoints that are of concern based on screening-level studies. For example, if a chemical is toxic to the brood but not to adults, then the Tier II study would focus on endpoints related to brood strength. Likewise, if a chemical is identified to be persistent, systemic, and can be brought back to the hive, then an overwintering design may be the most appropriate.

Typical designs of semi-field studies include a crop (or surrogate species such as *Phacelia spp.*) that is grown outdoors in an enclosed system with controlled or confined exposure. The test crop is grown under good agricultural practices, and the maximum application rate with the minimum application interval is used. The test crop provides the source of pollen and nectar. The design could also be structured to reflect a desired exposure system or foraging environment such as a mixture of crops and weeds or flowering margins, although such designs can introduce additional variables which may be difficult to control across replicates and treatments. A study can also be designed with a more artificial exposure system that makes use of spiked food sources to evaluate effects. As is evident, there are a range of different designs, and the actual test structure will depend on the specific question(s) asked in the risk assessment.

In the following section, aspects of the Tier II toxicity tests are discussed in terms of various methods which have been proposed to examine the toxicity of chemicals to bees under confined test conditions.

4.2.1 Toxicity directed to brood

Chemicals may be transported back to the hive thereby creating an exposure pathway for the brood within the hive. Sublethal concentrations of a toxic chemical in pollen and nectar may not impair adult foraging behavior and can allow foragers to collect contaminated food and return to the hive. This food may then be stored and residues may be translocated within the colony to areas where developing brood are present and serve as a route of exposure to those brood (Wu *et al.* 2011). The primary measurement endpoints for this type of study include egg abundance, larval abundance, pupal abundance, brood pattern, and emergence success (by following a cohort through development) and are typically expressed in terms of a statistically significant difference between treated and negative control hives. If studies have sufficient replication and multiple treatment groups are included, then the study design may be sufficient to yield NOAEC/LOAEC values.

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4.2.1.1 Semi-Field Tunnel Study (OECD 75; EPPO 170)

This study design is currently described by EPPO 2010 and OECD 2007 test guidelines. The test design employs a tunnel/tent in which small nucleus hives containing about 5,000 worker bees, brood in all stages, and stores of pollen and nectar are placed. A test crop is used at or near full bloom that is representative of high end exposure conditions for the given type of application method and rate. The hives are introduced in the tunnels 2-3 days before pesticide application (for foliar sprays) and hive condition is assessed while the bees acclimate to the enclosure. Pesticides can then be applied at peak bee foraging activity to maximize exposure to foraging bees. Hives are kept in the tunnel for a period of 7 – 10 days after the first pesticide application and allowed to forage on the treated crop. The hives are then removed from the tunnel and allowed to forage on untreated areas with continued monitoring (usually up to a total study period of 28 days, but sometimes longer). A schematic of the OECD semi-field tunnel study milestones and measurement endpoints is shown in **Figure 10**.

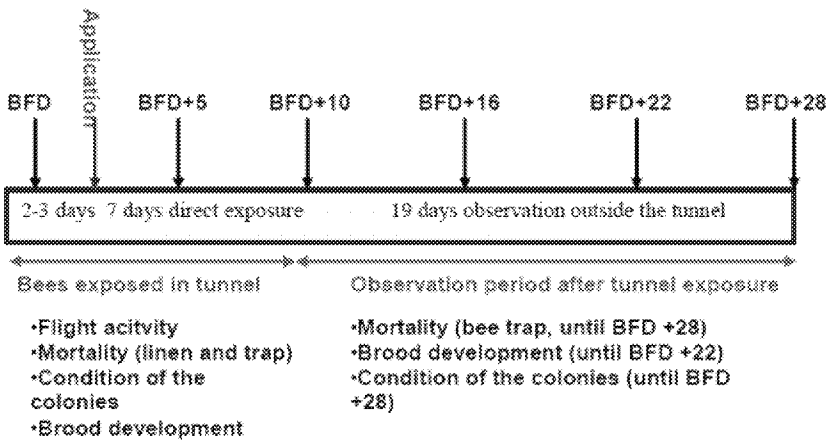

Figure 10. Schematic of Honey bee Semi-Field Tunnel Study Design (Source: OECD 2007). BFD = brood fixing day (*i.e.*, day colonies are introduced to the tunnel).

Measurement endpoints include brood condition (percent of brood area as eggs, larvae, capped brood) and associated indices (brood termination rate[11], brood index[12], and compensation index[13]), hive strength (percent of comb area covered with bees), queen health, adult bee mortality, and flight activity, which are measured at various points over the 28-d study. Results are evaluated by comparing the treated colonies with the controls (treated with water only). A reference chemical is also frequently

[11] Brood termination rate is determined by the number of brood in designated cells that do not reach the expected brood stage at a specified time.

[12] Brood index is calculated by scoring each cell development stage, totaling the scores and then dividing by the number of initially marked egg cells.

[13] Brood compensation index, considered an indicator of the recovery potential of the colony, is determined by calculating the number of identified cells where new brood cells are laid down after loss of the first effort.

tested to demonstrate that the application method used actually results in exposure to bees as well as the ability of the study to detect/quantify effects. Reference chemical selection is dependent on the expected mode of action of the pesticide under consideration, but should be a chemical with known toxicity to the hive. For pesticides that are expected to directly impact brood development, an insect growth regulator (IGR) is typically used (*e.g.*, fenoxycarb). For pesticides that are expected to elicit effects directly on adults (and indirectly on brood) through rapid 'knock down', a reference chemical that acts similarly (*e.g.*, dimethoate) is often selected.

The semi-field study design allows for an evaluation of the hive when it forages on an actual crop under a realistic application scenario. This enables the combined impact of lethal and sublethal effects on multiple castes of exposed bees to be ascertained at the hive level (*e.g.*, colony strength, brood development). These endpoints are more closely aligned to the proposed assessment endpoints (**Table 1**) compared to effects measured on individual bees. Furthermore, exposure to brood is realistic in the sense that larvae are exposed to the chemical in processed brood food rather than artificial matrices used in laboratory studies (*e.g.*, sucrose solution). Although typically used to assess foliar pesticide application methods, the semi-field tunnel study design is amenable to other application methods (*e.g.*, seed treatment of systemic pesticides) provided that exposure is adequately demonstrated (*e.g.*, plants are grown to full bloom and test chemical residues are quantified in pollen and nectar).

The semi-field tunnel design also contains limitations that relate to the known stress that the tunnels place on the foragers and consequently, the hives. Since the studies are conducted outside of a laboratory environment, they are subject to the vagaries of weather. As noted in the OECD guidance (OECD 2007), the test cannot be performed under adverse weather conditions; low temperatures can limit bee foraging activity and thereby affect potential exposure, while high temperatures can affect the extent to which nectar may be available as well as affect bee foraging activity. Because bees are not allowed to forage freely over large areas, the quantity and quality of food they are able to gather may be compromised compared to open field conditions (free foraging). Experience with these studies indicates that confinement stress on foraging bees, among other factors, often leads to adverse effects on egg, larval, and pupal abundance with increasing duration of confinement in the tunnels. As a result, hives can only be kept in tunnels for a relatively short period of time (7-10 days) to ensure adequate condition of controls for comparison. Even during this time, declines in control hive condition are commonly encountered. This relatively short exposure period introduces uncertainty when attempting to relate

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results to longer-term exposures that bees may experience in the field (*e.g.*, commercial hives being moved to multiple crops in a growing season). Furthermore, effects at other critical time periods of hive development (*e.g.*, overwintering) are not addressed, although such effects could be assessed if the post-tunnel observation period were extended and colonies were of sufficient strength to support overwintering.

As noted, the duration of semi-field studies has typically been limited to less than 2 weeks since bees/colonies begin to exhibit stress from being confined to tented enclosures/ tunnels for prolonged periods of time. However, while controlled exposure through confinement to a tunnel may be limited to ≤2 wks, observation periods for such studies may be extended for considerably longer periods of time provided that the colonies have sufficient forage. Colonies used in tunnel studies are typically reduced in size and may not have sufficient strength (in terms of overall numbers of bees) to collect the necessary stores to over-winter; however, it may be possible to build nucleus colonies up to sufficient strength following the enclosure period such that they could survive over-wintering. What is frequently done though is to transition to full field studies where full size colonies (~10,000 - 50,000 bees) are used to simulate more realistic conditions. Such studies are discussed in greater detail in the following section.

4.2.2 Field Feeding design

Although the feeding study does not involve enclosures/tunnels and bees are allowed to forage freely, the study is considered a semi-field study since test organisms are provided pesticide-spiked food, which does not occur in full-field studies.

The Oomen *et al*. 1992 method was developed in order to test for the possibility of adverse effects on the brood within a honey bee hive. The basic design uses 1 liter of a sucrose solution that is placed in a feeder near a full size honey bee hive. The sucrose solution is either a control without contamination or a solution spiked with a known concentration of a pesticide. The solution is provided as a food source, and once the hive has collected all of the solution, weekly measurements are taken on brood development within the hives; development is monitored by tracking the sequential development of brood through the use of acetate sheet overlays of the brood comb. In addition to the data on brood development, data on adult and brood mortality estimates can be obtained through counts of bees collected in dead bee traps placed at the entrance to the colony. Sublethal effect data on bee behavior and queen performance can also be included in the study. It is important to note that the hives are not

placed within tunnels but are allowed to freely forage. Further, the test solution represents a single exposure scenario that is expected to be consumed within one day. The test chemical concentrations are not meant to be reflective of environmentally relevant concentrations but rather may be exaggerated in order to screen chemicals for possible impacts to the brood when in a whole hive situation. An advantage of this type of study is that the hives are not confined within a tunnel as such confinement can put stress on the colony. The Oomen *et al.* 1992 method is, however, considered a qualitative assessment (OECD 2007) of effects to brood since the actual exposure to brood cannot be quantified.

EPA has considered further refinements in the semi-field study designs; however, these refinements have not been well vetted. The previous semi-field study methods have undergone review by the ICP-BR and the larger EU community and studies following these methods are becoming increasingly common in support of pesticide registrations in both the EU and to a lesser extent in the US. However, for the sake of completeness, other possible refinements in the semi-field study methods are discussed below and within the context of their strengths and limitations (summarized in **Table 22**). For systemic pesticides or those with a specific use pattern that can potentially contaminate food resources of bees, an alternative feeding design may further inform the risk assessment.

For this type of study, spiked food (pollen patties, sucrose solution, or both) can be fed *ad libitum* to full size honey bee hives of at least 10,000 bees over a specified duration of time and during a specified time of the year. Different concentrations of the chemical in food are used with the intent of establishing a NOAEC/LOAEC. The treatment levels are either selected from concentrations of concern that have been identified in the body of literature or by environmentally relevant concentrations found in targeted monitoring studies for residues in pollen and nectar. The design does not employ tunnels, so the bees are allowed to forage freely. While the potential exists for bees to forage in areas where other pesticides may be in use, test site selection is intended to minimize the potential for such exposure(s). Consequently, the test hives are placed in an area with minimal or no other crops present in order to minimize any exposure to other plant protection products. Consumption of the food sources provided to the hives are measured, as are the residues of concern (*i.e.*, parent and toxic metabolites) in the spiked food samples and hive stored pollen and honey to ascertain whether the spiked food is being stored. Measurements of residues potentially include other matrices such as bees (*i.e.*, to ascertain exposure of adult bees), royal jelly and brood food (*i.e.*, to ascertain exposure to the brood), and residues in bee-collected pollen and nectar from outside the hive (*i.e.*, to assess exposure to contaminants from sources

other than the spiked food). The measurement endpoints for this type of study are determined by the protection goals as well as remaining uncertainties highlighted by the lower-tier laboratory studies with individual bees as well as other lines of evidence (*e.g.*, open literature and/or incident data). Therefore, this type of study may be amenable to protocol modification to suit the current understanding of the pesticide chemistry, application methodology, and expected effect on the brood.

During a field-feeding study, exposure can be controlled to a greater extent than a full-field study through the use of pollen traps to limit incoming pollen and encourage feeding on the treated food source. As previously mentioned the enclosures used in the typical semi-field design introduce stress to the colony and affect the brood performance of the colonies in as little as 7 to 10 days. The open field feeding design allows a colony to freely forage thereby eliminating the stress of the enclosure. Furthermore, the study hives can be large enough to survive through overwintering, unlike the smaller nucleus hives typically used in the tunnel studies. The use of larger colonies enables the study to proceed over a longer duration than other semi-field studies in an enclosure. With this flexibility, the full-field feeding design can be used to evaluate a colony's response to either short-term or long-term exposure conditions to evaluate overwintering performance of the hives. Consequently, this study can address the uncertainties identified at the lower-tier levels or even at the initial stages of the Tier II toxicity assessment with other semi-field studies specifically when oral exposure is the pathway of concern.

The results of this study are intended to reflect the extent to which specific effects may occur in response to various pesticide levels in pollen and nectar when compared to measured residue levels in pollen and nectar from targeted monitoring studies. Assuming that targeted monitoring studies are available to design the feeding study or are available following the completion of the feeding study, these data would provide a measure of the level of residues in pollen and nectar from various crops. This feeding study could then provide a context for these residue data at the whole-hive level and reveal to what extent effects would be anticipated from the residue levels in the different crops via comparisons between the NOAEC and measured concentrations in pollen and nectar. The comparison between this NOAEC and the residue levels is not a quantitative comparison, but rather a means of understanding the potential impacts on the colony given realistic residue levels in pollen and/or nectar from various crops. No RQ's are generated with this study at the Tier II level, but the risk assessor could identify those residue levels above the NOAEC to be of concern; although such comparisons would have to account for the extent of the effects (*i.e.*, percent difference from controls) measured at the LOAEC.

These comparisons could then inform the risk assessor as to which crops or crop groups that were assessed in the tier II exposure analysis may be of most concern, and which crops could be excluded from further analysis based on the absence of an effect at similar residue levels. Consequently, this type of feeding study can reduce the number of crops for which a Tier III field pollinator study would be necessary or provide enough information at the Tier II level to remove the need for a Tier III study.

It is also important to note that there are a number of uncertainties with the field feeding study design (**Table 22**). First, the bees are allowed to freely forage, so consumption of the spiked food provided to the colony depends on a range of factors that may not be constant for all experimental situations. For example, if alternative forage is ample, then the bees may use less spiked sucrose solution or spiked pollen than when outside forage sources are scarce. Thus, this type of study, which does not use an enclosure, is subject to numerous sources of variability at the field level including weather, disease, parasites, and exposure to other pesticides present in the environment. Second, the feeding aspect is artificial and may not accurately mimic typical foraging behavior by honey bees or the stability of the test material in such matrices under natural conditions (*e.g.*, full sunlight for photolytic compounds). Third, use of an internal spiked food source can confound efforts to use foraging behavior as a measurement endpoint. A lack of foraging on the spiked food source could be interpreted as either intoxication, or alternatively as avoidance of the contaminated food item or simply that an alternative source of food was preferred. Fourth, the performance of the colonies has not been evaluated in relation to long-term continual use of the artificial food sources. Finally, as the purpose of the study is to identify a NOAEC, the study should be designed to provide sufficient statistical power to detect the desired level of biological significance, based on the potential impact to the assessment endpoints of concern. However, at this time, a level of biological significance for survival, growth, and reproduction at the whole hive level is unknown.

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Table 19. Comparison of Tier II Semi-Field Study Designs

Test Type	Study Objectives	Typical Endpoints	Strengths	Limitations	Utility in Risk Assessment	Tier III Study Refinements[a]
Semi-field (Tunnel) OECD 75; EPPO 170	Toxicity to developing brood under whole-hive exposure conditions to pesticide treated crop in semi-field tunnels.	• Brood development • Brood termination rate • Brood index • Brood compensation index • Colony strength • Queen health • Pollen & nectar storage • Adult mortality • Foraging/flight activity • Abnormal behavior • Worker longevity	• Whole hive response is quantified in addition to different bee castes/life stages • Impact on developing brood are assessed • Multiple exposure routes can be assessed (direct contact, oral exposure) • Actual pesticide application methods and rates are assessed	• Exposure duration is limited (~7 days of direct exposure) due to tunnel stress • Long-term effects (overwintering) are not typically addressed • Exposure may not represent realistic crop exposure scenarios (crop type, temporal and spatial scales)	• Identify a range of potential whole hive effects of short-term exposure scenario based on worst-case realistic maximum applications to a specific crop • Evaluation of effects to brood and adult castes related survival, growth, reproduction, honey production, and pollination services	• Allows a longer duration of study • Assess long-term whole hive effects following chronic exposure to a test crop • Refine the effects characterization by evaluating the impacted measurement endpoints without the added stress of the tunnel
Semi-field, Open (feeding)	Toxicity to developing brood under whole-hive, free foraging exposure conditions via repeated exposure to spiked diet (*e.g.*, pollen cakes)	• Brood development • Brood termination rate • Brood index • Brood compensation index • Colony strength • Queen health • Pollen & nectar storage • Adult mortality	• Free foraging design reduces stress on colonies compared to tunnels • Exposure can be evaluated over long time periods • Oral dose can be controlled and quantified • Whole hive response is quantified in addition to different	• Uncertainty regarding the extent to which bees will consume stored spiked food. • Residues on alternative sources of pollen/nectar • Exposure may not represent realistic crop exposure	• Evaluation of a range of potential effects to adults and/or brood based on uncertainties from lower tier studies or other semi-field studies • NOAEL based on concentrations within in-hive food sources (surrogates for pollen and nectar) • Ability to interpret	• Natural conditions allow for realistic exposure pathway • Ability to assess whole hive effects when foraging activity is a known endpoint of concern • Refine the effects characterization for crops whose residue levels fall between the LOAEL and the NOAEL

		• Foraging/flight activity Overwintering • Worker longevity	bee castes/life stages • Impact on developing brood are assessed • Potential for known exposure to residue levels measured in pollen/nectar • NOAEL independent of crop in order to be broadly comparable for comparison with all crops • Does not require large field plots	scenarios (crop type, temporal and spatial scales) • Does not capture effects to foraging behavior on contaminated food sources	residue levels in pollen and nectar from targeted field residue monitoring studies	

[a] This column provides that potential rationales for progressing to a tier III field study in order to address any remaining uncertainty.

4.3 Tier III Effects Assessment

4.3.1 Refinement of Tiers I and II

The results of the field study should be linked to the regulatory concerns identified by risk managers. It is also important to note that the full field study is intended to represent a highly refined level of assessment and one that addresses specific uncertainties regarding the potential for colony-level effects. The information obtained from a field study must be considered in the context of the full body of information available for a chemical; therefore, the risk assessment must consider the entire weight of evidence. As discussed previously though, a field study may initially be intended to provide a general understanding of whether a chemical results in adverse impacts to an entire colony. If there is an effect in the screen, a definitive study may be needed to quantify the magnitude of the effect in order to better inform risk management decisions. As risk management decisions are made, various forms of mitigation may be considered and those efforts may reduce potential risks. The effects of mitigation should be evaluated through each step of the refinement process such that field studies do not need to be repeated to better account for such efforts.

Although this section is included under the effects characterization, Tier III effects assessments are intended to represent highly refined studies that address specific uncertainties and/or risks identified in lower-tier studies. Current U.S. testing requirements to support pesticide registrations indicate that if certain conditions are met, refined testing may be required. According to the 40CFR158, field testing for pollinators (OCSPP Guideline 850.3040; USEPA 2012*c*) using a technical end-use product (TEP) is required if data from other sources indicate potential effects on colonies particularly if those effects involve endpoints other than acute mortality. Field testing may also be required if toxicity of residues on foliage tests (*e.g.*, OCSPP Guideline 850.3030; USEPA 2012*b*) indicate that the pesticide exhibits extended residual toxicity (*i.e.*, pesticides with residues which remain toxic to >25% of the organisms tested for periods >8 hrs) or if data from studies with terrestrial arthropods other than bees indicated potential chronic, reproductive or behavioral effects. A field study serves as a means of addressing uncertainties raised in lower-tier studies conducted either in a laboratory or under restricted conditions inside an enclosure/tunnel or with an artificial food source and can be useful in examining the effects of pesticides with extended residual toxicity. However, a full field study is intended to represent realistic application conditions whereby the test substance is applied to a specific crop on which bees are foraging freely without the use of an enclosure and whereby test colonies are primarily exposed through

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residues carried back to the hive by the worker bees. Consequently, a full-field study is also the most resource intensive (relative to studies used for the Tier I and II assessments) to conduct in terms of both time and money; these studies can also be the most difficult to interpret given the level of associated variability. Therefore, they can represent a significant investment to regulatory agencies in terms of the resources required to review these studies, but these studies also provide context relative to other lines of evidence. The weight attributed to these studies as to how they influence regulatory decisions varies widely across regulatory authorities; however, well conducted studies can be useful in determining, under actual use conditions and typical foraging activity of bees, whether a compound represents a significant risk to bees.

Given the expense to conduct full-field studies and that they are typically conducted on the basis of results from lower-tier studies, the availability of appropriate guidance is important to ensure that the studies will be useful in a regulatory context. In the following sections, study design elements for full field pollinator studies are discussed. As with the semi-field study, the length of the full-field study is dictated by the specific questions and uncertainties which the study is intended to address. Although the exposure component of semi-field studies is frequently limited to a maximum of 2 weeks, observation periods can extend beyond that time. However, with full-field studies, even the exposure period can be extended to reflect actual use conditions and for those studies which are examining over-wintering success, the study duration can range from several months to a year. It is important to note that increasing the duration of a study can also increase the likelihood of confounding effects such as disease, pests, nutritional deficits, and weather.

The results of the field study should be linked to the regulatory concerns identified by risk managers. It is also important to note that the full-field study is intended to represent a highly refined level of assessment and one that addresses specific uncertainties regarding the potential for colony-level effects. The information obtained from a field study must be considered in the context of the full body of information available for a chemical; therefore, the risk assessment must consider the entire weight of evidence. As discussed previously though, a field study may initially be intended to provide a general understanding of whether a chemical results in adverse impacts to an entire colony. If there is an effect in the screen, a definitive study may be needed to quantify the magnitude of the effect in order to better inform risk management decisions. As risk management decisions are made, various forms of mitigation may be considered and those efforts may reduce potential risks. The effects of mitigation

should be evaluated through each step of the refinement process such that field studies do not need to be repeated to better account for such efforts.

4.3.2 Design of a Field Study for *A. mellifera*

The actual design of the field study should be based upon both the uncertainties identified at the lower tiers as well as protection goals that are identified at the problem formulation phase of the assessment. For example, if a protection goal is to maintain pollination services, then the field study can be tailored to address pollination activity based on specific adverse effects (*e.g.*, impacts to behavior, adult bee longevity, *etc.*) identified in the lower tier registrant-submitted studies and/or open literature and the properties of the chemical (*e.g.*, extended residues on foliage, systemic distribution in plants, *etc.*). Information contained in the targeted monitoring studies examining residues in pollen and/or nectar coupled with effects observed in acute toxicity tests with adults/larvae and/or colony level effects from Tier II tunnel/feeding studies can inform the design of the field study by focusing future studies on the primary routes of exposure or crops that represent the worst case exposure scenarios and on measuring particular effects. The aim of the field study is to test the potential effects of a specific chemical under actual use conditions and compare the results with those obtained in laboratory and semi-field studies in order to better characterize potential risks to pollinators from the use of a chemical.

A full-field study should be designed based upon specific questions identified from the lower tiers to reflect a range of exposure scenarios. Although a single field study may not address all uncertainties and/or use scenarios, it should be designed to represent relatively worst case conditions that are considered protective for other uses. In focusing the study design on specific concerns, crops that are good sources of both pollen and nectar, and have a high treatment rate, in addition to being highly attractive to honey bees, may serve as an ideal target crop. Currently, the EPA has a guideline for conducting the pollinator field study, which is under 40CFR158 as guideline number 850.3040 (USEPA 2012*c*). Similarly, the EPPO guideline 1/170 (4) (EPPO 2010) also addresses pollinator field study guidelines. The EPA field pollinator guideline does not provide much detail, but rather provides flexibility for designing a study based on the needs of the risk assessment. However, EPPO 1/170 (4) (EPPO 2010) provides more detail that can be used to design a field pollinator study with honey bees in terms of experimental conditions (*e.g.*, selection of the crop, placement of colonies relative to the treated field,

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size of the colonies, plot size), application of treatments (*e.g.*, positive/negative controls, timing of application, rates) and mode of assessment (*e.g.*, timing and frequency of assessments).

Although intended as guidance for conducting field studies with mammals and birds, the EPA Guideline 850.2500 (Field Testing for Terrestrial Wildlife; USEPA 2012*d*) provides very useful information on study design elements to consider for field testing. This guidance discusses the importance of clearly articulating the purpose of a field study in advance since its purpose should dictate how the study is ultimately designed. According to the guidance, the purpose of a field study is to either refute the assumption that risks to wildlife will occur under conditions of actual use of the pesticide or to provide some quantification of the risk that may occur. To address these concerns, field studies can be either screening or definitive. If laboratory toxicity data on individual bees and/or colony level data are available from semi-field studies, these data coupled with measured residue data from target monitoring studies to estimate risk and a screening field study may be appropriate to determine if impacts occur at the colony level under actual use conditions. In this case, the screening field study would be intended to confirm or refute an assumption of risk to honey bee colonies. If a screening-level study and/or incident data indicate that impacts are occurring, or if other available data (reliable open literature data) suggest that deleterious effects have occurred or are likely, the guideline recommends that the study design should be quantitative, thereby evaluating the magnitude of the impacts in a definitive study. Therefore, screening field studies may provide "pass/fail" information as to whether colonies are being adversely affected by an application; however, definitive studies should provide information on the magnitude of effects (*e.g.*, acute mortality of forage bees, the extent of effects on brood production/survival).

An important consideration in the selection of a study site is the geographic area. Although representation of a wide range of biogeographic areas is ideal, it may not be practical. Therefore, to keep the number of study sites practical while still accomplishing the purpose of the study, site selection should be based on those areas that are likely to represent the greatest risk or highest concern, are representative of an area/crop which bees are likely to frequent in the highest density, and consider the extent to which untreated alternative forage is available and can be minimized. The extent and nature of alternative forage in the vicinity of the study site(s) should also be considered in site selection.

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Additionally, the number of study sites is also an important consideration and OCSPP Guideline 850.2500 (USEPA 2012*d*) provides guidance on methods used to determine necessary sample sizes depending on the level of confidence required in the study result. However, there can be practical limitations to the number of study sites and as noted in the guidance, the number of sites may be reduced if study site selection is biased toward worst case, *i.e.*, conditions which are considered likely to result in relatively high exposures. Statistical analysis should be conducted for all field studies; therefore, field studies should also determine the power of the test to adequately assess the endpoints relative to the appropriate level of biological significance. A power analysis should accompany each protocol proposal in order to determine the number of hives necessary for the conduct of the study. In addition, the final report should contain a power analysis to determine if the appropriate level of statistical power has been achieved.

Another important consideration is the size of the study sites. The size of the study site must ensure adequate exposure of bees to the test material and reduce the opportunity for bees to forage on untreated sites. The OCSPP Guideline 850.2500 (USEPA 2012*d*) notes that the study should never be less than an individual field in which a crop is planted. Distance between study sites is also an important design element. Although the guidance indicates that sites should be separated adequately to ensure independence, it notes that this is dependent mainly on the range of the species. Distance between treated and control sites is important given that honey bees can forage for considerable distances depending on the availability of food. Winston 1987 reports that the median foraging radius of honey bee colonies in agricultural areas is a few hundred meters and is 1.7 km in forested areas; however, distances up to 11 km (7 miles) have been reported when no other competing food sources are available. Again, there can be practical constraints to achieving ideal distances to ensure absolute independence of study sites.

As noted, test conditions should resemble the conditions likely to be encountered under actual use of the end-use product; in most instances the pesticide should be applied at the maximum treatment rate and reapplication frequency specified on the label. If more than a single method is specified on the label, then the method that maximizes exposure of bees should be used.

Based on the information contained in the aforementioned guidelines, a field study for honey bees in general should be conducted under actual use conditions in which a typical end-use product is used at

its maximum application rate, frequency, and method as described on the label for a specific crop. The evaluation of the hive should ideally proceed for a minimum of 50 days based on the lifecycle of the honey bee within the colony. For the EPPO 2010 study protocol, the following description applies. The study is conducted on a crop grown outdoors without enclosures; consequently the primary exposure route is to foragers. The crop is subject to good agricultural practices, and the bees are allowed to freely fly and forage. The design should reflect a realistic foraging environment and exposure system in the field where the pesticide is to be applied. Hives (each with a minimum of ~10,000 bees) are placed within or at the edge of treated or control flowering plots and monitored for a specified period of time depending on the concerns of the chemical (*e.g.*, systemic compound that is persistent in hive food stores). Because hives may differ in size, colonies should be distributed as equitably as is possible between treatments. The colonies should be in position approximately 2–3 days before the trial in order to acclimate to the study site. If the chemical is a foliar spray, treatments should be applied when the test crop is in full flower during the daytime when bees are demonstrated to be actively foraging on the test crop. Hives should be queen-right (*i.e.*, have a single functional queen), use sister queens (*i.e.*, common maternal source), and be of similar strength at the start of the study. The EPPO guidance notes that during spray applications, the test hives should be protected from spray drift. Equipment should be free of other chemicals, and the hives should have low incidences of disease and parasites, though it is nearly impossible to use hives completely free of disease/pests. Unlike semi-field enclosure studies, in the case of a field study, a toxic reference standard (*e.g.*, dimethoate) is usually not suitable. The test system includes the treated plot and an untreated control and ideally should be suitably separated to minimize the extent to which bees from either group can interact. Although very desirable, the EPPO guidance suggests that replication is often not feasible because of the requirements for adequate separation of replicates to ensure independence. The potential endpoints that can be measured in a field study include the following:

- Colony strength
- Weight of the hive
- Pollen, honey, nectar stores
- Mortality at the hive
- Mortality of drones and pupae
- Mortality in the crop
- Presence of the same queen
- Ability of the colony to replace the queen (requeen) naturally
- Foraging activity in the crop
- The returning of foraging bees
- Behavioral abnormalities

- Residues in pollen, nectar, pollen pellets, wax, honey, bee bread, and dead bees
- Assessment of the brood, including an estimate of adults, the area containing cells, eggs, larvae, and capped cells
- Disease resistance or pest levels
- Worker longevity
- Over-wintering success

These endpoints represent the range of endpoints available for measurement in full-field studies; however, actual measurement endpoints should be dictated by the stated objective of the study and the specific uncertainties it is intended to address. The study protocol must articulate the objective of the study, its measures of effect and how these data will be collected and analyzed. In terms of protocol development, any number of endpoints may be selected for measurement and analysis. However, the endpoints selected should be based on those that are of concern as identified in the lower tier or open literature studies and the fate properties of the chemical. Therefore, one field study likely would not measure all of the endpoints identified above. Field studies may also focus on different time periods during a hive's lifecycle in the year for more rigorous sampling depending on the chemical. For example, if overwintering is a concern, then the period of intense sampling could be immediately before and after overwintering. The extent to which a colony is manipulated during a study can affect the study outcome; therefore, consideration must be given to the extent to which a colony must be opened and the nature of the measurement endpoints that will be collected at that time to determine the most efficient and least invasive means of collecting those data.

4.3.3 Analysis and Interpretation of Effects

The analysis and interpretation of full field studies must consider a variety of factors which include how and the extent to which the bees/colonies are exposed and the nature of the effect(s) both in terms of the statistical and biological significance. Additionally, these studies are intended to be refinements based on lower-tier studies of effects (at both the individual bee and colony level) and based on lower-tier exposure estimates (based on modeled or measured residues). Therefore the design, conduct and analysis of full-field studies must be considered in the context of studies which served as a foundation for requiring such studies. Given the shorter duration of lower-tier studies and their oftentimes exaggerated exposure conditions, the full-field studies offer an opportunity to understand the potential effects of a pesticide on bees/colonies under actual use conditions and to determine whether effects observed in lower-tier studies occur to an extent which would result in sufficient adverse effects to warrant mitigation. Understanding the nature of the measurement endpoints and their relationship to assessment endpoints along an AOP should influence the extent to which impairments can or should be weighted. The previous discussion of sublethal measurement endpoints, where the relevancy of a particular effect to the overall impaired survival of the colony must be considered as well as the extent to which a colony may recover from such effects. As discussed earlier, the determination as to whether the likelihood and magnitude of an adverse effect (*i.e.*, risk) is sufficient to warrant mitigation is dependent on a number of factors and these decisions extend beyond risk assessment.

In the following sections, some of the factors associated with the analysis and interpretation of full field studies are discussed. These sections and the factors discussed therein are not intended to be exhaustive; however, they are intended to provide an indication of factors that should be considered in advance of conducting studies in order to facilitate their eventual analysis/interpretation.

4.3.3.1 Exposure

The analysis of the data obtained from field studies involves comparisons of the hives on treated plots with those on untreated (negative control) plots. It can be difficult to evaluate the effect of the test chemical within a full field study because the design allows for bees to forage freely and to obtain food both on site and off the treated site. In studies with limited bloom density on the treated crop and extensive alternative forage in the vicinity, bees may actually forage very little in the treated crop and this may lead to an underestimation of potential effects on the hive due to reduced exposure or alternatively to an inability to detect treatment effects due to control contamination.

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Consequently, it is important to obtain accurate estimates of exposure to the test crop. This estimate can be informed by several lines of evidence. First, pollen traps affixed at the entrances of the hives force pollen off of the legs of a percentage of foragers when they return to the hive. This pollen can then be analyzed for residues in addition to the floral origin of the pollen as well. Second, qualitative measures of foraging activity, potential quantitative measures of the return of forager bees (*e.g.*, measured through the use of radiofrequency identification tags; RFID), and quantitative measures of bloom density can provide information on the potential attractiveness of the crop of interest. Third, freshly stored nectar and pollen or stored bee bread and honey can be measured in the hives to determine the level of residues that the bees are storing for consumption or processing prior to feeding of the queen and brood. When nectar is collected, some pollen from the flowers may also be collected on the bees and mixed with the nectar when it is stored. So in addition to sampling residues in freshly stored nectar, pollen could potentially be obtained from within this collected nectar to determine the origin of the nectar as well. Measures of residues in the blossoms would provide a comparison of stored concentrations with those collected from the blossoms.

The estimates of exposure can be difficult to obtain, however. If older comb is used in the study, it is possible that the hives will enter the study with reserves of bee bread and honey, or deposited pollen and nectar. If this is the case, exposure may be difficult to ascertain as the bees could consume older stores first. Another possibility is the sampling of the residues inside the hive may not be representative of the residue levels from the incoming stored food as the food reserves are spatially and temporally diverse.

Typical methods of statistical analysis for the measurement endpoints include measures of central tendency (*e.g.*, means) and measures of dispersion (*e.g.*, variance, standard error) and comparison of treatment means to those of controls. All of the toxicity endpoints are typically analyzed separately using statistics, primarily through group comparisons with a single variable to test for differences from the control group. For example, a decrease in brood may be related to a number of factors, including the amount of food resources coming into the hive. Worker mortality, foraging intensity, disease and parasite prevalence, and honey production, all of which may impact the hive level food resources, would be analyzed individually in order to help explain possible changes in brood abundance. However, this approach requires the collection of many additional endpoints and may also be stressful to the hive due to the invasive nature required by the collection methods. Therefore, the field study should focus on

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only the parameters of interest as the field study design should address the potential for an effect as opposed to the mechanistic causes of an effect.

Time trends are not routinely reported, but they can be qualitatively evaluated in order to identify changes in the hive condition over time. These should be evaluated carefully however, as hive parameters naturally change over time. Therefore it is essential that control colonies perform adequately in order to provide a concurrent measure of performance.

4.3.4 Biological Significance

Higher-tier studies can provide information on a wide-range of effects and depending on the study design and the amount of variability associated with a particular measurement endpoint, there can be uncertainty regarding the interpretation of whether a statistically-significant effect is biologically significant. Low variability may enable the statistical detection of subtle changes in a measurement endpoint; however, there can be uncertainty whether the extent of the change is of sufficient magnitude to be of concern. Conversely, there may be uncertainty whether an effect, that is not statistically significant but occurs in a consistent and elevated frequency in treated colonies compared to negative controls, is biologically significant. Additionally, time series analyses may reveal short-lived effects that may be statistically significant and of sufficient magnitude to be of concern; however, if the colony is able to compensate for these effects, there is uncertainty regarding the extent to which such effects should influence a risk assessment. Therefore, reconciling statistical and biological significance can be a challenge.

4.3.5 Experimental Design Weaknesses

The preceding discussion of factors associated with the analysis and interpretation of full-field studies is further informed by considering some of the limitations that have been associated with previous field studies submitted in support of pesticide registrations. In the following sections, issues associated with the level of exposure to the pesticide of interest as well as to other pesticides with which the test bees/colonies may have come in contact are discussed. Also, colony size, distance between study plots, colony health (pests and diseases) and study duration are discussed in terms of some of the issues associated with previously submitted studies. All of these factors can potentially confound efforts to interpret study results and can quickly render full field studies of low utility to risk assessment.

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4.3.5.1 Level of Exposure

Honey bees as the test species within a field study are able to forage freely. Consequently, the study has limited ability to control where the bees forage since it does not confine them to only the test crop as a food source. Considering the number of factors that affect the foraging intensity on the test crop (*e.g.*, attractiveness of crop to bees, weather), this aspect of the study design is one of the most difficult to address at the full field study level.

A field study that was conducted with a relatively new class of insecticides/miticides serves as an example of the challenge related to honey bee foraging behavior. One of the representative chemicals was evaluated in foliar applications to citrus and the subsequent effect on honey bee brood and the hive was assessed. The study used a single orchard for the treatment and a single orchard for the control; therefore, neither group was replicated. The treated and control orchards were 0.53 ha (1.3 acres) in size. The hives were equipped with a minimum of food at the start of the study; however, the study report did not state how much food (*i.e.*, stored pollen and honey) the hives were allowed to carry into the test at the initiation of the study. Data on the amount of stored food at test initiation is essential as it provides insight into the potential for alternative sources of untreated food. The study report also stated that the orchards were isolated and not in close proximity to other flowering crops or extensive blooming weeds, though it was not mentioned how far from the test orchards the study authors had accounted for.

This study included pollen identification and examined the source of pollen entering the hives. A comparison of the sources of pollen is shown in **Tables 20** and **21**. These data suggest differences between control and the treated plots as well as differences between hives within a treatment and the differences over time within the same hives. During the peak period of foraging on the control plot, 24% of pollen, on average, originated from citrus. However, in the treatment hives, only 2.2% of the pollen on average originated from citrus. The majority of pollen entering the treatment hives came from a variety of other plant species. While the amount of pollen originating from citrus changed over time in both the control and treatment group, this relationship of higher level of citrus pollen entering control hives was consistent over the course of the sampling period. This study reveals that the assumption of equal foraging of treated and untreated plots does not always hold and highlights the importance of pollen identification analysis. It also reinforces the uncertainty in conclusions from the field study design when bees are allowed to freely forage as there is a high level of variability over time given likely

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differences in bloom intensity (surrogate for attractiveness) and differences between where different hives forage. It also highlights the question as to what is a worst-case scenario for the foraging on a specific crop. This study provides an example of an important consideration for a field study, *i.e.*, that the field study should have a sufficiently attractive test crop to meet the foraging needs of the test hives.

Table 20. Results of a pollen identification analysis (palynology) in a field study on citrus with a test chemical – treated plots.

Sampling Date	DAA2[1]	Hive Number	Pollen Source [%]					
			Citrus sp.	*Hypecoum* sp	*Quercus ilex*	*Helianthemum* sp.	*Olea europaea*	Other[2]
18 Apr 2008	+7	1	4	-	16	64	-	16
18 Apr 2008	+7	2	1	22	-	52	-	25
18 Apr 2008	+7	3	0	-	-	-	-	100
18 Apr 2008	+7	4	9	-	-	72	-	19
18 Apr 2008	+7	5	<1	-	-	92	-	8
18 Apr 2008	+7	6	1	-	-	86	-	13
18 Apr 2008	**+7**	**Mean**	**2.7**	**3.7**	**2.7**	**61**	**-**	**30.2**
25 Apr 2008	+14	1	0	-	53	27	-	20
25 Apr 2008	+14	2	1	-	-	78	-	21
25 Apr 2008	+14	3	0	-	48	39	-	13
25 Apr 2008	+14	4	2	-	25	64	-	9
25 Apr 2008	+14	5	<1	-	26	68	-	6
25 Apr 2008	+14	6	9	-	26	49	-	16
25 Apr 2008	**+14**	**Mean**	**2.2**	**-**	**29.7**	**54.2**	**-**	**14.2**
07 May 2008	+26	1	1	-	59	22	-	18
07 May 2008	+26	2	<1	-	60	-	33	7
07 May 2008	+26	3	<1	-	37	38	-	25
07 May 2008	+26	4	<1	-	45	25	12	17
07 May 2008	+26	5	1	-	35	47	9	8
07 May 2008	+26	6	1	-	38	15	40	6
07 May 2008	**+26**	**Mean**	**1**	**-**	**45.7**	**24.5**	**15.7**	**13.5**

[1]DAA2: days after the second application of the test substance
[2]Anacardiaceae, Asteriaceae, Lamiaceae, Papaveraceae, Cistaceae, Fabaceae, Brassicaceae, Fumariaceae, Resedaceae, Rosaceae, Salicaceae, Pinaceae, Oxalidaceae, Lauraceae, Euphorbiaceae, Mimosaceae, Rhamnaceae, Ranunculaceae, Boraginaceae, Clusiaceae, Convolvulaceae

Table 21. Results of a pollen identification analysis (palynology) in a field study on citrus with a test chemical – control plots.

Sampling Date	DAA2[1]	Hive Number	Pollen Source [%]					
			Citrus sp.	*Hypecoum* sp	*Quercus ilex*	*Helianthemum* sp.	*Olea europaea*	Other[6]
18 Apr 2008	+7	1	35	24	22	-	-	19
18 Apr 2008	+7	2	5	28	21	-	-	45
18 Apr 2008	+7	3	14	-	35	37	-	14
18 Apr 2008	+7	4	3	30	-	20	-	46
18 Apr 2008	+7	5	19	9	25	17	-	30
18 Apr 2008	+7	6	3	14	-	-	-	83
18 Apr 2008	**+7**	**Mean**	**13.2**	**17.5**	**17.2**	**12.3**	**-**	**39.8**
25 Apr 2008	+14	1	5	34	13	7	-	41
25 Apr 2008	+14	2	31	-	24	-	-	45
25 Apr 2008	+14	3	21	-	19	-	39	21
25 Apr 2008	+14	4	49	-	-	10	-	41
25 Apr 2008	+14	5	23	-	19	13	-	45
25 Apr 2008	+14	6	13	25	31	-	-	31
25 Apr 2008	**+14**	**Mean**	**23.7**	**9.8**	**17.6**	**5.0**	**6.5**	**37.3**
07 May 2008	+26	1	3	-	-	-	62	35
07 May 2008	+26	2	5	-	-	-	41	54
07 May 2008	+26	3	5	-	-	-	81	14
07 May 2008	+26	4	19	-	-	-	55	26
07 May 2008	+26	5	2	-	-	-	54	44
07 May 2008	+26	6	18	-	14	-	23	45
07 May 2008	**+26**	**Mean**	**8.7**	**-**	**2.3**	**-**	**52.7**	**36.3**

[1]DAA2: days after the second application of the test substance

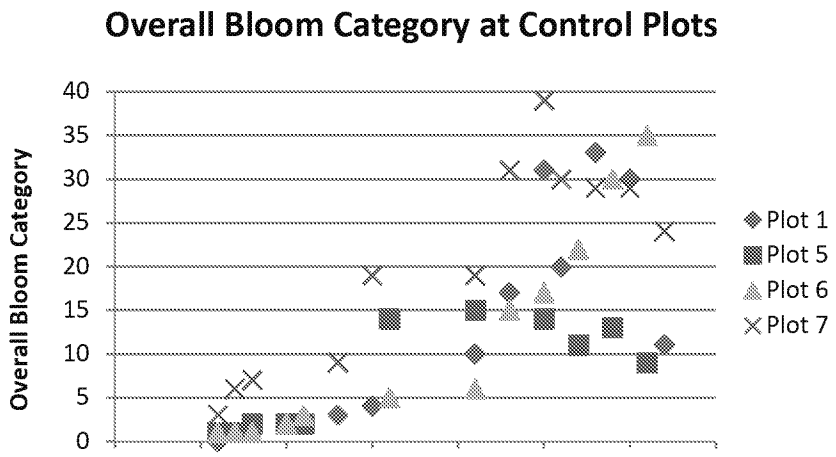
[2]Anacardiaceae, Asteriaceae, Lamiaceae, Papaveraceae, Cistaceae, Fabaceae, Brassicaceae, Fumariaceae, Resedaceae, Rosaceae, Salicaceae, Pinaceae, Oxalidaceae, Euphorbiaceae, Rhamnaceae, Ranunculaceae, Boraginaceae, Clusiaceae, Asphodelaceae, Plantaginaceae, Scrophulariaceae

The bloom intensity of the crop and level of alternative forage can impact the amount of foraging on the treated crop as well. Fields of a given crop with determinate blooming (*i.e.*, having a defined bloom period) will roughly bloom at the same time. However, peak bloom intensity may vary, in some cases by up to a couple of weeks. **Figure 11** illustrates a field study conducted with a test chemical on citrus in Argentina. Four treated citrus orchards and four control citrus orchards were separated by 3 km or more from each other. In this study, the authors conducted an assessment in which the blooming on the different plots was categorized on different days during the study. The study authors estimated the number of blossoms on each plot on 2 randomly chosen branches of 5 impartially pre-selected citrus trees within the orchard that were also "in proximity" to the hives. The study authors did not provide a distance between the selected citrus trees and the hives. A bloom category was assigned to each branch, and the category values of all branches were summed to provide an overall bloom category for a plot (possible range from 0 to 40). The bloom category was based on the number of flowers in various

stages from no completely open flowers to flowers that were in full bloom and completely open. The categories were as follows based on the number of completely opened flowers:

- Category 0: no completely open flowers per branch
- Category 1: >25 completely open flowers per branch
- Category 2: 25<x<50 completely open flowers per branch
- Category 3: 50<x<75 completely open flowers per branch
- Category 4: >100 completely open flowers per branch

As **Figure 11** shows, the plots varied in bloom intensity over the course of the study, and they also varied by treatment. The control plot appeared to bloom earlier than the treatment plots, perhaps due to site characteristics and/or the varieties of citrus, which also varied in the study, *i.e.*, the control plots were primarily Okitsu versus the W. Murcott or Lanelate varieties in the treated plot.



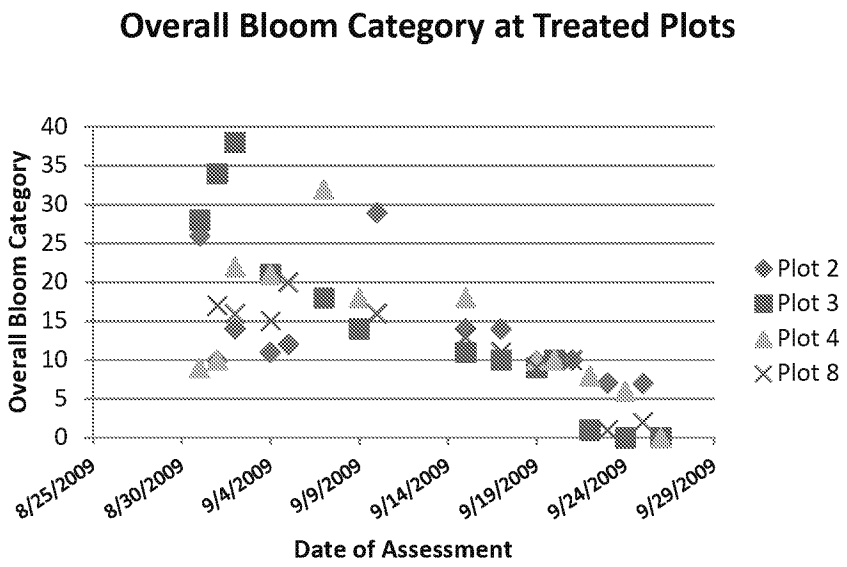


Figure 11. Classification of blooming intensity in a field study with citrus showing differences in blooming timing.

Hives were placed in the field at the same time, so differences in bloom intensity can affect the attractiveness of the forage crop, or shift the focus of honey bee foraging to alternative forage sources. Given the range of foraging distance for a honey bee, bees may utilize variety of alternative sources if the test plot is inadequate in terms of area or extent of bloom.

Another consideration is that colonies may contain significant reserves of untreated pollen and honey that serve as food as identified earlier in the section related to exposure. Furthermore, all or part of the test substance-containing nectar and pollen that are collected by foragers may be stored in the colony and may not be used immediately. Therefore, during a study with a relatively short duration, the extent to which residue-containing food is consumed may be an uncertainty. Furthermore, the test substance in pollen and/or nectar may break down to less toxic, or potentially more toxic, metabolites as it is stored and not immediately consumed. This aspect of storage also presents an uncertainty for the exposure of the colony to a test chemical.

Relating exposure to weather, bees will not forage during rain storms or when temperatures are too low for bee flight. This may affect the activity and the strength of the hives, as well as exposure to the test chemical. Furthermore, in temperate areas, an unseasonably cold winter may also affect loss of hives in both control and treated groups due to overwintering stress and confound the ability of the study to detect treatment effects.

4.3.5.2 Exposure to Other Pesticides

Agricultural fields are rarely separated in space and time from other pesticides. These products may be applied to the same crop as part of grower standard practice, or they may be applied on neighboring fields on which honey bees may forage or from which the pesticides may drift into the test or control plots of a full field study. In the case of persistent plant protection products, these chemicals may remain on the test field or in the adjacent field well after the application. These products may then be collected by honey bees and brought back to the hive thereby serving as a source of exposure to other chemicals or even the chemical under study.

4.3.5.3 Colony size

The size of the test colonies is an important consideration for the design of the field study. According to EFSA recommendations, a population of 10,000 individuals is identified as the minimum size. However, this size is not representative of a normal colony during the spring and summer seasons, which is typically between 20,000 (spring) and 60,000 or more (June - July) individuals. Considering the minimum size of 10,000 individuals, this size corresponds to the beginning of hive development at the end of the over-wintering period just before the hive starts rapid expansion in the early spring. Exposure from different types of applications may occur not only in the early spring (*e.g.*, foliar spray to tree crops) but also later in spring and during summer (*e.g.*, seed treatment to corn or canola) when larger-sized colonies are more representative.

EFSA 2012 also notes several points related to the advantages of different sizes of the hives for the field study. Larger populations may be more efficient honey producers and produce more honey on a per bee basis while using less per bee over the winter. Thus, a larger colony with more foragers and in-nest bees could provide a better observation of the effects of the pesticide because of the higher number of bees involved. However, EFSA also notes that smaller colonies may be more sensitive due to reduced resilience to replace foragers with nurse bees and a smaller brood area.

4.3.5.4 Separation of treatment from control plots

The separation of the treated plot from the control plot is essential considering the foraging range of the honey bee. For control and treatment plots with inadequate separation where honey bees can forage freely, bees from control plots may forage on treated plots and bees from treated plots may forage on control plots thereby bringing contaminated food into the control hives. Conversely, treatment plot hive

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bees may forage in control plots as well, thereby decreasing the extent of exposure. This movement of control and treatment bees between plots can confound the interpretation of the field study.

In one case study, a chemical was applied as a seed treatment in a 130-day study under open field conditions at four test sites. Each site contained one 1-ha field planted with canola seed that had been treated with the test substance and one 1-ha field with untreated seed to serve as a control. Each of the treated and control fields were separated by at least 250 m. Four honey bee colonies were placed in the middle of each of the eight fields (n=32) during a 3-week canola bloom period beginning on July. According to the study authors, no other flowering crops or corn grown from seed treated with the test substance were planted within a 1-km radius of any of the test plots. In addition, the authors also stated that the availability of alternative forage within 1 km of the test plots was minimal. While potential forage crops such as soybean, corn, and alfalfa were located within 1 km of some of the test plots, none were in bloom while the honey bee colonies were in the canola test plots.

The residue analysis of bee-collected nectar and pollen, as well as honey and beeswax, indicated that bees from the control and treatment groups were cross-foraging in both the control and treatment fields. The majority of samples (>75%) collected had no detectable residues, whether from colonies in treated or control fields. Residues of the test material were detected in treatment plot nectar at concentrations ranging from 0.521 to 2.24 ppb, while concentrations ranged from 0.535 to 0.969 ppb in control hives when detected. These detections in the control hives indicate that bees from these hives likely foraged on test plots, which may be a function of inadequate separation between control and treated test plots or because the forage in some control fields was of lower quality (due to insect damage and lower rates of plant emergence). It is also possible that treated bees were able to forage in control plots, which contributes to the uncertainty associated with inadequate separation between control and treated test plots.

4.3.5.5 Parasites and Pests

There are a number of parasites and diseases that afflict honey bee hives. These include varroa mites, the microsporidian *Nosema spp.*, bacteria (*e.g.*, American foulbrood; *Paenibacillus larvae*), small hive beetle (*Aethina tumida*), wax moth (*Galleria mellonella*), fungi (*e.g.*, chalkbrood; *Ascosphaera apis*), and viruses (*e.g.*, deformed wing virus and Israeli acute paralysis virus; IAPV). Some of these pests/diseases, in particular *varroa* mites and *Nosema spp*, can affect some of the endpoints of concern as well as the survival of the hives themselves. It is also nearly impossible to eliminate infection/infestation completely

from hives. Therefore, field studies are likely to have some level of infection/infestation involved in the conduct of the study. Treatments for the pests may occur before the conduct of the study, though this should not impact the performance of the test hives. If the disease or parasite occurrence is too high, the ability of the study to detect potential treatment effects may be limited. Furthermore, the condition of the test hives after any hive maintenance treatments should be evaluated in the acclimation phase of the test hives prior to exposure to the test substance.

4.3.5.6 Duration of the study

The duration of the study should be based on the persistence of the chemical of interest. However, as EFSA discusses in their report (EFSA 2012), the maximum duration of the experiment in the guideline EPPO 170 (28 days) is not adapted to assess the effects of systemic and persistent pesticides on all the categories of bees inside the colony. Considering that the life of a bee is about 50 days or more during spring and summer (21 days of pre-imaginal development and at least 28 days for the adult life), and several months in winter, a longer duration of assessment may be required depending on the chemical of interest.

4.3.6 Suggested modifications

EPA, PMRA and CalDPR have identified a number of ways to improve upon the basic design of a full field study. These suggestions are based on field study reviews by EPA, PMRA and CalDPR and recommendations proposed in EFSA review of scientific methods for evaluating risk concerns to pollinators (EFSA 2012). These additions or changes are as follows:

- Increase the sizes of the test plots. The size of the plots should reflect typical North American agricultural areas. The size of the areas should meet the nutritional requirements of the hive for the duration of the study, in addition to reflecting typical agricultural crop acreage in North America. It is recognized however, that the bloom period of the test crop may be short relative to the overall study period, but the study design must consider that the nutritional requirements of the study colonies are met.
- Statistical analysis should be conducted for all field studies; therefore, field studies should also determine the power of the test to adequately assess the endpoints relative to the appropriate level of biological significance. A power analysis should accompany each protocol proposal in order to determine the number of hives necessary for the conduct of the study. In addition, the final report should contain a power analysis to determine if the appropriate level of statistical power has been achieved. While this is the ideal, it is recognized that there can be practical

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constraints to achieving a desired statistical power. Actual study designs for higher tier studies are case-by-case and are intended to address specific uncertainties identified in lower-tier and/or open literature studies.

- Measures of exposure are essential to the interpretation of the field study. Consequently, the study should measure residues in incoming pollen (through the use of pollen traps) and/or nectar (sampling of the bee honey stomach), measure residues in stored pollen and nectar pre-exposure and at various times post-exposure, measure bloom intensity and duration of that intensity, and identify sources of pollen and nectar (through pollen trapped in in-coming nectar).
- Limit disease/pests using new hive equipment or source bees from low pest incidence sources.
- A field study should provide for adequate separation of treated and control plots. However, climatic and landscape variations may preclude greater distances between plots depending on these variations over varying geographic scales. According to EFSA (EFSA 2012), the field tests should use areas with similar environmental conditions, where possible with at least 4 – 6 km (2.5 – 3.7 miles) between treated and control plots.
- The duration of the study should match the concern of the chemical. According to the EFSA 2012 scientific opinion, the colonies that are used in the experiment should be monitored for a period of time covering all of the flowering period and beyond and last at least two brood cycles. When residues are persistent, monitoring should continue through winter to after the over-wintering period. For the over-wintering monitoring, the test and control colonies should be placed in an area far from intensive agriculture in order to limit the exposure of hives to pesticides other than from the test crop. This aspect of the design is necessary not only for the treatment groups but also for the control groups as exposure to the latter may lead to an underestimation of the toxic effect of the test material.
- Finally, EFSA 2012 proposes other recommendations to control or increase exposure of bees to test material. The study should use highly attractive crops and regular assessments of foraging. Pollen traps should be used to determine the pollen levels coming in. The pollen should be analyzed not just for color but for floral origin (microscopic examination of pollen) as well. The pollen should be subjected to a residue analysis to confirm that the bees near the treated field have been exposed and to confirm that the control colonies have not been contaminated. Other means of assessing or controlling exposure include the following:

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- Foragers on the flowers – a quantitative assessment of observations of foraging bees on the flowers of the treated or control field, in combination with a qualitative assessment of the behavior of the foraging bees
- Foragers on the flight back to the hive– a qualitative assessment of observations of foraging bees returning to the hives, including aggressive interactions with the guard bees
- The in-nest bees – quantitative assessment of hive brood following OECD 75 guidance document (OECD 2007)
- Residue analysis of in-hive bees (in particular nurse bees)/dead bees from bee trap
- Evaluation of the storage of food and the residues contained in the food stores (bee bread, honey)
- Removal of food stocks

Implementation of the above additions or modifications would be part of the study development phase. As has been the approach to date for pollinator field studies, EPA as well as PMRA and CalDPR have requested that registrants submit protocols for such studies prior to study initiation. This white paper proposes to continue this approach in order to promote efficiency and increase the potential for success given the cost of pollinator field studies in terms of time and resources.

Because of the expense involved in conducting and reviewing full field pollinator studies, this paper has gone into detail on study design elements to consider when conducting such tests. However, the ultimate design of the study will be dictated by the specific uncertainties it is intended to address. While these studies are conducted in response to concerns identified in lower tier studies, the full field study is ultimately intended to address whether risks estimated using lower tier exposure and effect data occur under actual use conditions. The results of full field studies can have an important effect on the understanding of lower tier data and of the potential risks.

5 Risk Characterization

Risk characterization represents the final step in the risk assessment process and is intended to integrate estimates from the exposure and effect characterizations to derive quantitative estimates of risk (USEPA 2000). Similar to the process used for evaluating potential risks to other taxa and as discussed earlier in this document, screening-level (*i.e.*, Tier I) risk assessments typically rely on point estimates of potential exposure and effects which are considered conservative. The risk characterization is also intended to articulate assumptions, limitations and uncertainties associated with the risk assessment. In clearly articulating each step of the risk assessment and its underlying process/assumptions, it assures that the process is transparent and the assessment itself is clear, consistent and reasonable. That is to say that the risk manager and any stakeholder can understand how risk conclusions were arrived at, that the approach used is consistent with processes identified in EPA guidelines, that it could be readily repeated and that relatively similar conclusions would be reached given the same underlying data and assumptions.

The risk characterization consists of two components, *i.e.*, risk estimation and risk description. In risk estimation exposure and effects data are compared in the form of a ratio (*i.e.*, RQ) and within the context of regulatory levels of concern. In the risk discussion section, multiple lines of evidence are considered to further qualitatively describe potential risks. These lines of evidence can include incident data and studies reported in open literature which have been determined to be of sufficient quality. The risk description section then evaluates lines of evidence, in terms of the adequacy and quality of data as well as the degree and type of uncertainty, used in support of the risk assessment. To the extent possible, the risk description should attempt to articulate the nature and intensity of effects as well as their temporal (*e.g.*, whether recovery occurs) and spatial scale. In addition, this risk description should evaluate the effects of potential mitigation on risk estimates.

As depicted earlier in **Figures 2** and **3**, the proposed risk assessment process is intended to be iterative. At a screening level (Tier 1) RQ values are estimated and if they exceed LOCs, estimates of exposure may be refined based on measured rather than modeled residue levels. If LOCs are still exceeded based on laboratory-derived toxicity endpoints for individual bees, higher tier refinements can be considered to determine whether the whole colony may be affected under semi-field (Tier II) or full-field (Tier III) testing conditions. In the following sections the risk estimation and risk description components of risk characterization are discussed.

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5.1 Risk Estimation

RQ values represent the ratio of point estimates for exposure (numerator) and effects (denominator). As discussed in the exposure characterization section, the screening-level risk assessment relies on exposure estimates for foliarly applied compounds based on T-REX (dietary exposure) and Koch and Weisser 1997 (contact exposure) while dietary exposure estimates for soil treatments are estimated using the Briggs' model and seed treatments are based on ICP-BR's 1 mg a.i./kg default (**Table 22**). As with all risk assessments, one of the most relevant questions early in the assessment is whether exposure to bees is likely. This question represented one of the earliest phases of the risk assessment process depicted earlier in **Boxes 2a** and **2b** of **Figures 2** and **3** of **Section 1**. For those products for which bees are likely to be exposed, the screening-level (Tier 1) assessment considers potential risks from both contact exposure (depicted in **Figure 2** of **Section 1**) and ingestion of residues (depicted in **Figure 3** of **Section 1**). Effects estimates for use in adult honey bee RQ values rely on the acute contact (**Box 4a** of **Figure 2** of **Section 1**) and oral LD_{50} values for young adult bees (**Box 4b** of **Figure 2** and **Box 4a** of **Figure 3** of **Section 1**) while potential risks to bee larvae are estimated using the LD_{50} value for larvae (**Box 4c** of **Figure 2** and **Box 4b** of **Figure 3** of **Section 1**). Although the risk assessment process developed through the SETAC Pellston workshop (Fischer and Moriarty 2011) recommended the use of a NOAEC value for determining acute risk to larvae, these values are not routinely measured in the current acute toxicity tests, as the studies have regression-based designs and do not typically have sufficient replication to support the hypothesis testing needed to support the development of a reliable NOAEC. Additionally, as discussed in the effects characterization section of this white paper, chronic toxicity tests have not been sufficiently vetted at this time for consistently measuring such effects; therefore, while this white paper discusses the role of such measurements in the risk assessment process, suitable tests have yet to be developed to allow the calculation of chronic RQ values.

Table 22 summarizes the exposure and effect estimates used in developing the Tier 1 screening-level RQ values for individual adult bees and larvae for foliar spray applications, soil applications, seed treatments and tree trunk applications. The appropriate exposure values and effect endpoints would be used in **Boxes 4a**, **4b** and **4c** of **Figure 2** of **Section 1** and **Boxes 4a** and **4b** of **Figure 3** of **Section 1**. The resulting RQ values would then be compared to the LOC which is discussed in the next section.

Table 22. Summary of exposure and effect estimates used in deriving risk quotients for Tier I risk assessments.

Measurement Endpoint	Exposure Route	Exposure Estimate [a]	Acute Effect Endpoint	Chronic Effect Endpoint
Foliar Applications				
Individual Survival (adults)	Contact	(2.7 μg a.i./bee)*AR	Acute contact LD_{50}	None
Individual Survival (adults)	Diet	(32 μg a.i./bee)*AR[c]	Acute oral LD_{50}	Acute adult oral NOAEL (effects to survival or longevity)**
Brood size and success	Diet	(13 μg a.i./bee)*AR[c]	Larval LD_{50}**	Acute oral NOAEL for larvae (effects to survival, amount of brood) [d]
Soil Treatments				
Individual Survival (adults)	Diet	(Briggs EEC [b])(0.29 g/day)	Acute oral LD_{50}	Acute adult oral NOAEL (effects to survival or longevity) [d]
Brood size and success	Diet	(Briggs EEC [b])(0.12 g/day)	Larval LD_{50}	Acute oral NOAEL for larvae (effects to survival, amount of brood) [d]
Seed Treatments				
Individual Survival (adults)	Diet	0.29 μg a.i./bee	Acute oral LD_{50}	Acute adult oral NOAEL (effects to survival or longevity)[d]
Brood size and success	Diet	0.12 μg a.i./bee	Larval LD_{50}	Acute oral NOAEL for larvae (effects to survival, amount of brood) [d]
Tree Trunk Applications				
Individual Survival (adults)	Diet	0.29*(μg a.i. applied to tree/g of foliage)	Acute oral LD_{50}	Acute adult oral NOAEL (effects to survival or longevity) [d]
Brood size and success	Diet	0.12*(μg a.i. applied to tree/g of foliage)	Larval LD_{50}	Acute oral NOAEL for larvae (effects to survival, amount of brood) [d]

AR = application rate (in lbs a.i./A)
[a] Based on food consumption rates for larvae and adult worker bees as described in Appendix 1.
[b] Exposure to soil treatments is based on the modified Briggs plant uptake model; whereas seed treatment exposure is based on the assumption of 1 mg/kg concentration of a pesticide in pollen and/or nectar
[c]See T-REX manual for method of calculating the exposure value when there are multiple applications.
[d]Test is currently under development.

5.1.1 Levels of Concern (LOCs)

Historically, the EPA has not quantified risk to honey bees using the deterministic RQ-based approach used for other taxa. In the current approach, the potential hazard to bees is qualitatively based on the outcome of acute contact toxicity tests. This has typically resulted in recommended label language similar to that contained in the Environmental Hazards section of the Label Review Manual for honey bee hazard statement (USEPA 2012). Although the language is intended to address potential acute effects, the guidance indicates that label language to reduce potential chronic hazards to bees will be dealt with on a case-by-case basis.

One method which has been used to determine whether the potential risk from pesticides is sufficiently low such that either additional refinements in risk estimates or mitigation is not needed, is through the use of monitoring data. In the European Union, hazard quotients (HQ) derived by dividing the maximum application rate for a sprayed product by the acute median lethal dose (LD_{50} based on oral or contact toxicity) are compared to a value of 50. If the HQ is less than 50, the product is considered to be safe for bees (Thompson and Thorbahn 2009, Mineau *et al.* 2008) unless the product is an insect growth regulator. The value of 50 is based on an analysis of more than 20 years of honey bee colony incident data from the Wildlife Incident Investigation Scheme (WIIS), which were suspected to be associated with pesticide applications and represented a total of 234 poisonings (Mineau *et al.* 2008). The WIIS is a voluntary reporting system in the United Kingdom, and it relies on beekill incident reports from beekeepers and other interested organizations. Information on pesticide use was obtained from the UK Pesticide Usage Survey data which are obtained from crop surveys conducted every 2 – 6 years over a 21-year period. Based on this analysis, it was determined that HQ values <50 posed negligible risk of hive mortality incidents. The paper by Mineau *et al.* 2008 acknowledges the potential for under-reporting of beekill incidents in the field, *i.e.*, acute bee mortality is not pronounced at the hive itself and goes unobserved and that the predictive models developed in their analysis may not account for all of the factors that could influence the likelihood that the use of a pesticide would result in acute mortality.

Although the risks to honey bees have not been quantified in past pesticide assessments conducted by EPA, assessments conducted in response to litigation under the Endangered Species Act have required the quantification of potential direct and indirect effects to federally-listed threatened or endangered species (hereafter referred to as "listed"). For the purposes of these assessments, OPP developed interim guidance (dated May 15, 2007) recommending that acute RQ values are compared to a level of concern (LOC) of 0.05 to determine whether the use of a chemical is likely to result in adverse acute effects to listed terrestrial invertebrate species (CFR40 2012*b*)[14]. According to the interim guidance, the LOCs for birds, mammals and aquatic species were based on 1975 regulations for the enforcement of FIFRA (40 CFR Part 154: 49005, 49007 and 49016). For birds and mammals, the following regulation applied:

"[I]t can be estimated that a dose or exposure of 10 times lower than the LD_{50} or LC_{50} would be expected to lead to mortality rates of about 0.01 percent (assuming a dose-response slope of 4.5) and 4 percent (assuming a dose-response slope of 2), respectively. A dose 5 times lower than the LD_{50} or LC_{50} would be

[14] For the current list of Federally endangered and threatened arthropod species, see: http://www.fws.gov/endangered/species/us-species.html

expected to lead to mortality rates of about 0.1 percent and 10 percent, respectively. These values were used as a basis for selecting safety factors of 5 to 10X for setting the classification criteria for protecting wildlife." (CFR40)

A safety factor of 10, which results in an acute LOC of 0.1, was established for birds and mammals; however, an acute LOC of 0.05 was set for aquatic species as an added measure of protection since these organisms were considered confined to a particular location and did not have the same opportunity as birds and mammals to avoid exposure. This same rationale was applied to terrestrial invertebrates as well.

In the following section, an approach is described for developing LOCs with which to compare the acute and chronic RQ values for bees proposed in this white paper. The approach follows a similar process used previously but takes greater advantage of insect pollinator-specific toxicity data, including slope information from acute toxicity studies with bees, and the extent to which bee colonies may be able to sustain/accommodate acute mortality events.

Although EPA typically relies on a default acute dose-response slope of 4.5 (95% confidence interval: 2 – 9), an analysis of the slope values for registrant-submitted honey bee acute contact and oral toxicity data indicates mean (95% confidence interval) values of 3.93 (1.32 – 9.56) and 3.40 (1.33 – 7.18) respectively (**Table 23**). These values are relatively close to the default value used for other taxa. Based on various slope values and the likelihood of individual mortality estimated using the EPA Microsoft® Excel-based Individual Effect Chance Model (IEC; Version 1.1), which allows for such calculations by entering slope estimates, various LOCs have been derived (**Table 24**). Depending on the slope of the dose response curve from acute contact and oral toxicity studies and on the level of mortality, LOC values range from 0.02 for 1% mortality with a slope of 1.4 to an LOC of 0.64 for 10% mortality based on a slope of 6.6.

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Table 23. Statistics associated with slopes of acute oral and contact toxicity data available in EFED's toxicity database for pesticides.

Statistic	Contact	Oral
number	69	26
min	0.8	1.2
max	18.2	8.5
average	3.9	3.4
5th percentile	1.3	1.3
10th percentile	1.5	1.4
25th percentile	2.1	2.1
median	3.0	3.2
75th percentile	4.9	4.4
90th percentile	6.6	5.0
95th percentile	9.6	7.2

Table 24. Levels of concern associated with different slope values and different levels of mortality in worker bees.

Slope	Comment	1% mortality	5% mortality	10% mortality
1.4	10th percentile slope (oral)	0.022	0.067	0.12
1.5	10th percentile slope (contact)	0.028	0.08	0.14
3	Median slope (contact)	0.17	0.28	0.37
3.2	Median slope (oral)	0.19	0.31	0.40
5	90th percentile slope (oral)	0.34	0.47	0.55
6.6	90th percentile slope (contact)	0.45	0.56	0.64

As discussed throughout this document, the loss of individual bees is an important indicator of toxicity and useful for estimating risk; however, the protection goals identified in the problem formulation section of this white paper as they relate to honey bees depend on the proper functioning of the colony itself. Discussions with beekeepers who have reported incidents to the EPA indicate that colonies can (depending on the time of year) sustain sudden losses of 30 – 40% and recover, although losses at such levels may compromise honey production for a period of 6 weeks. If the colony were to experience such losses during a period of dearth (*i.e.*, when pollen and nectar sources may not be readily available to foraging bees), the effects could be more protracted and result in substantially reduced food reserves and potentially increased vulnerability to disease. The reasonable level of mortality on which to set the LOC is based on the amount of mortality test guidelines allowed in control groups (*i.e.*, 10%). The proposed Tier I method includes a LOC of 0.4 for acute exposure. This value is based on a median slope of 3.2 and a limit of 10% mortality (**Table 27**) for both estimating risk from either an acute oral or contact exposure of larvae or adult bees. Although chronic toxicity test guidelines are not currently available for bees, the evaluation of potential chronic risk for other taxa is typically conducted by

comparing the NOAEC from such studies to EECs to ensure that that ratio is below 1, *i.e.*, the LOC for chronic risk is set to 1.0.

With reference to the decision trees depicted in **Figures 2** and **3** of this document, the screening-level assessment compares RQ values to LOCs (**Box 5** of **Figures 2** and **3**) to determine whether there is a presumption of minimal risk or whether additional refinements may be needed. Based on the preceding discussion, the LOC to which the screening-level acute RQ values would be compared is 0.40. For pesticide uses where RQ values exceed the LOC, refinement options for both exposure and effect numbers have been discussed in this white paper. These include the use of measured residues values rather than modeled estimates to decrease the magnitude of the RQ's numerator; such refinements in the exposure estimates (Tier II exposure; **Box 9a** of **Figures 2** and **3**) are considered to be part of the Tier I risk assessment process since the result of such refinements enables the calculation of an RQ value (**Box 6** of **Figures 2** and **3**). Refinements to Tier I effects endpoints are based on the understanding that the Tier I studies are on individual bees and the assessment endpoint for honey bees is at the colony level. Therefore, when Tier II semi-field studies (depicted in **Box 9b** of **Figures 2** and **3)** and/or Tier III full-field studies (depicted in **Box 12** of **Figures 2** and **3**) are available, they should be evaluated to determine whether effects to individual bees translate into adverse effects at the colony level, the nature of those effects and the duration of such effects. Placing RQ values into context through the use of additional lines of evidence is conducted in the risk description step of the risk assessment and is discussed in the next section.

5.2 Risk Description

Screening-level estimates of risk at Tier I are intended to be conservative and therefore protective for taxa for which the test organism serves as a surrogate. As with other taxa, the reliance on conservative estimates of exposure and effects for bees needs to be appropriately characterized. The risk description section is intended to evaluate all of the lines of evidence available for a particular chemical in conjunction with the quantitative RQ values. Information from open literature (including peer reviewed journals and "grey literature", such as published dissertations) and registrant-submitted studies which meet the standard for inclusion in risk assessments can be used to qualitatively describe potential adverse effects which are expected to result from the use of the chemical and can represent useful sources of information on the toxicity of pesticides to non-*Apis* bees as well. Given the nature of the current guideline study designs, the risk estimation section places considerable emphasis on survival as

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a measurement endpoint; however, it is recognized that the absence of statistically significant levels of mortality in either individual bees or in colonies cannot be construed as the absence of adverse effects (Thompson 2002). Thus, this section should include a discussion of sublethal effects which may have been reported. Additionally, relevant ecological incident data from the EPA EIIS and the National Pesticide Information Center (NPIC 2012) and through the Canadian PMRA Pesticide Product Information Database (Health Canada 2012) should be used as lines of evidence to support risk estimates. Risk assessors should evaluate the multiple lines of evidence in determining whether additional data should be recommended.

As discussed throughout this document, the risk assessment process is intended to be iterative and requires that risk assessors and risk managers collaborate to identify potential risk management options which will dictate the risk assessment process. At the end of Tier I testing, if the pesticide is determined to be potentially hazardous to bees two options are available. One option is to proceed to Tier II for further effects testing (depicted as **Box 9b** of **Figures 2** and **3**) or refinement of exposure estimates (depicted as **Box 9a** of **Figures 2** and **3**). The other option is to employ risk mitigation measures (**Boxes 8** and **11** of **Figures 2** and **3**). As noted in the discussion above, these measures usually seek to limit bee exposure via the use of label restrictions. Where mitigation measures result in reduced loading (*i.e.*, lower application rates or number of applications), the effect of reduced loading on exposure estimates should be re-evaluated through available models. Since the lower proposed rates may not have higher-tier monitoring data available with which to calibrate estimated exposure values, it may not be possible to determine the extent to which modeled estimates reflect actual exposure levels.

Where the focus of Tier I effects testing is on individual bees, Tier II effects testing focuses on the whole hive. Tier II and III testing may be conducted if concerns are identified in Tier I testing or from other sources such as published literature. Tier II and III testing is more complex and endpoints identified relate to impacts at the whole hive level. Thus, mitigation based on this level of testing will be more refined and will reflect specific issues identified in the tests. However, in general the Tier II (**Box 9b** of **Figures 2** and **3**) tests are the first to examine potential effects to the whole colony and provide an opportunity to understand whether effects observed on individual bees under laboratory test conditions extend to the whole colony. In considering the higher tier colony-level toxicity tests, the risk assessment should include a description of the nature and duration of the anticipated effect and whether there is

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evidence to support that protection goals may not be attained as a result of the proposed use of a pesticide.

At this time, the proposed risk assessment method for bees includes qualitative use of Tier II and Tier III effect studies to characterize potential risks to the whole colony that may result from the proposed use of a compound. Since these studies are typically conducted at maximum proposed application rates and on vulnerable crops (*i.e.*, crops which are considered to be particularly attractive to bees and serve as good sources of both pollen and nectar), they are not used quantitatively to generate RQ values. Studies conducted at Tier II under semi-field (tunnel/feeding) studies may not fully reflect the effects of chemicals on honey bees which are free foraging and able to select from a broader range of food sources; however, these studies provide additional information with which to characterize effects on the whole colony and better enable risk assessor/risk managers to determine whether additional data and/or mitigation measures are necessary.

The risk assessment process is intended to integrate multiple lines of evidence. The process used for evaluating these multiple lines of evidence has not been specifically articulated for honey bees; however, it has been described in a draft guidance developed for evaluating the weight of evidence for the Endocrine Disruptor Screening Program (EDSP) Tier 1 screening to identify candidate chemicals for Tier 2 testing (USEPA 2010*b*). In the draft guidance, it indicates that the body of available data is taken into account for consistency, coherence, and biological plausibility. This analysis not only applies to the outcome of guideline studies but also other scientifically relevant information, which in the case of risk assessments for bees would include targeted residue monitoring studies, open literature studies, and incident reports. More specifically, the evaluation of individual studies includes the characterization of:

- Quality of data and the extent to which effects can be replicated within a laboratory and across different laboratories;
- nature of the effect(s) seen in the study(ies) (*e.g.*, were the effects seen in studies persistent or transient changes; were sublethal changes or adverse outcomes);
- dose- and time- dependent changes, if available;
- strengths and limitations of results;
- number and type of effects induced and magnitude, and severity of effects;
- consistency, pattern, range, and interrelationships of effects observed across studies, species, strains, and castes/sexes;
- conditions under which effects occur (*e.g.*, dose, route, duration);
- understanding of MOA and biological plausibility of responses; and,
- specificity and sensitivity of the effect(s).

Effects observed in studies are considered in the context of both statistical and biological significance; the level of confidence is determined by the strengths as well as the limitations and uncertainties associated with the study.

Since the overall risk assessment integrates information across multiple assessment tiers, key questions which should be asked include but are not limited to the following.

- How do exposures used to quantify effects compare to those expected in the field? Specifically, are the duration, frequency and magnitude of exposures used to quantify effects likely to be higher or lower than those expected in the real world settings?
- How do the spatial scale of exposures used to quantify effects compare to those expected in the field?
- What is the nature of effects observed across multiple Tiers (*e.g.*, duration, magnitude, frequency)? Are observed effects consistent across multiple Tiers? If not, why not?
- What are major sources of uncertainty and bias in exposure and effects assessment across different tiers? To what extent can additional studies reduce this uncertainty?
- Are observed effects consistent with what is known about the pesticide's mode of action?
- What is the biological and statistical significance associated with effects observed in the various studies? How closely are the observed effects related to the assessment endpoints?
- To what extent do other lines of evidence (*e.g.*, incident reports, open literature studies) support or detract from the exposure and effects reported in submitted studies?

Although this list is not intended to be exhaustive, it demonstrates how information from the different tiers of the assessment must be integrated to provide reasonable understanding of how the exposures and effects evaluated at the individual bee and whole colony level relate and how risk estimates are affected by refinements.

The risk characterization draws on multiple lines of evidence on both exposure and effects (to individual bees and the colony) based on guideline exposure and toxicity studies as well as open literature studies and incident data. In doing so, the risk characterization should relate back to the risk hypothesis articulated and conceptual model depicted in the problem formulation to inform the risk manager of whether the analysis has led to a presumption of minimal risk or not and identify uncertainties associated with either conclusion. Ideally, there should be congruence between the different lines of evidence considered in the assessment; however, where there are differences and/or seeming contradictions, the characterization should attempt to provide some explanation or identify these as uncertainties.

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One area of uncertainty is the extent to which the risk assessment may be protective for non-*Apis* bees. Some of the considerations associated with the extent to which honey bees serve as reasonable surrogates for non-*Apis* bees have been discussed previously; however, a more detailed discussion follows.

5.3 Consideration of Non-*Apis* Bees in Ecological Risk Assessment

The focus of this white paper and proposed risk assessment process on the honey bee, *A. mellifera*, reflects two important factors: 1) honey bees are considered the most important pollinator in most regions of the world from both a commercial and ecological perspective;[15] and 2) standardized test methods for evaluating exposure and effects of chemicals in a regulatory context are much more developed with the honey bee compared to non-*Apis* bees (EFSA 2012). Nonetheless, within North America alone, there are an estimated 4,000 species of bees (Michner 2007) and this number rises to more than 20,000 worldwide (Fischer and Moriarty 2011). Several species of non-*Apis* bees are commercially managed for their pollination services, including bumble bees (*Bombus spp.)*, leaf cutting bees (*Megachile rotundata*), alkalai bees (*Nomia melanderi*), and blue orchard bees (*Osmia lignaria)*, and the Japanese horn-faced bee (*O. cornifrons*). Importantly, a growing body of information indicates native bees (in addition to other insect pollinators such as flies, moths, butterflies, beetles, wasps, and ants) play an important role in crop and native plant pollination, besides their overall ecological importance via maintaining biological diversity. Although the proposed method does not include a formal risk assessment process that is specific to non-*Apis* bees, the need for such a process is clear owing to potential differences in sensitivity and exposure compared to honey bee. The purpose of this section is to briefly summarize the aspects of non-Apis bees which require additional consideration in the context of the current proposed risks assessment process for honey bee. Potential avenues for modifying the proposed risk assessment process are also explored.

5.3.1 Biological and Ecological Considerations

Several aspects of the biology and ecology of non-*Apis* bees lead to important differences in the route and extent to which they may be exposed to pesticides compared to honey bees. These aspects have been recently reviewed (EFSA 2012, Fisher and Moriarty 2011) and are summarized here briefly.

[15] According to Tautz, J. (2008), approximately 80% of the world's flowing plants are pollinated by insects and 85% of these by honey bees. In all, the list of flowering plants pollinated by honey bees includes 170,000 species.

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Specifically, many non-*Apis* bees are smaller in size and thus, would receive a higher dose on a contact exposure basis (*i.e.*, greater surface area to volume ratio) via intercepting droplets of sprayed pesticide. Most non-*Apis* bees are solitary nesting species[16] and therefore, loss of a single nesting adult would have a much greater consequence on reproduction (at least for that nest) compared to the loss of a single adult foraging honey bee. Furthermore, the foraging range of non-*Apis* bees tends to be much smaller than that of honey bees. As a consequence, non-*Apis* bees that occupy areas adjacent to treated fields may be exposed to pesticides at a higher proportion of their foraging area compared to honey bees, which can forage over long distances (~7 km) in which they are more likely to encounter untreated forage areas. For ground nesting bees, exposure via direct contact with soil (and inhalation of volatile soil-applied fumigants) may be a major route of exposure unlike that for the honey bee. Soil and leaf material are known to be used extensively by some non-*Apis* bees for nest construction, which may lead to different types of exposures (*e.g.,* prolonged contact exposure with contaminated residues on treated foliage).

5.3.2 Exposure Considerations

The above discussion indicates that differences in biology and life history among *Apis* and non-*Apis* bees will lead to differential exposure to pesticides. To investigate the extent to which exposure estimates for honey bees may serve as a surrogate for non-*Apis* bees, comparisons were made in the daily consumptions rates of pollen and nectar available from the literature as compiled by EFSA (2012). Although there are a number of uncertainties associated with these consumption estimates, the data in **Tables 25** and **26** suggest that proposed food consumption rate for adult honey bee workers (292 mg/bee/day) is similar to that for adult bumble bee (210-402 mg/bee/day) and is greater than that of adult female European mason bee and alfalfa leaf cutting bees (45-193 and 110-165 mg/bee/day, respectively). Food consumption rates estimated for 5-day old honey bee larvae (120 mg/bee/day) are greater than rates for larvae of the other non-*Apis* bees (7.8-83 mg/bee/day) shown in **Table 26**. This suggest that the proposed Tier 1 exposure assessment for food consumption by adult honey bees would be representative (and somewhat protective) for adults these particular non-*Apis* bees.

[16] Colonies of the social non-*Apis* bees (*e.g.*, bumble bees and stingless bees) tend to be smaller than honey bees.

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Table 25. Comparison of Oral Exposure to Pollen and Nectar for adult *Apis* and Non-*Apis* Bees

Species	Nectar consumption rate (mg/bee/day)*	Pollen consumption rate (mg/bee/day)	Total food consumption rate (mg/bee/day)
Honey bee worker *(A. mellifera)*	292	0.04	292
Bumblebee *(Bombus spp.)*	183-372	27-30	210-402
European mason bee *(Osmia cornuta)*	45-193	na	45-193
Alfalfa leaf-cutting bee (*Megachile rotundata*)	110-165	na	110-165

na = not applicable

Table 26. Comparison of Oral Exposure to Pollen and Nectar for larval *Apis* and Non-*Apis* Bees

Species	Male/ female	Nectar consumption rate (mg/bee/day)*	Pollen consumption rate (mg/bee/day)*	Total food consumption rate (mg/bee/day)
Honey bee *(A. mellifera)*	Female	117	2.7	120
Bumblebee *(Bombus spp.)*	unknown	60	22-23	82-83
European mason bee *(Osmia cornuta)*	Female	1.8	16.3	18
	Male	1.1	9.5	11
Alfalfa leaf-cutting bee (*Megachile rotundata*)	Female	6.2	3.1	9.3
	Male	5.2	2.6	7.8

* from stored provisions

As discussed previously, non-*Apis* bees are expected to be exposed to pesticides via soil and plant material used for nest construction. For the European mason bee, contact exposure to mud by adult females has been estimated at 200 – 400 mg/bee/day. Similarly, contact exposure of alfalfa leaf cutting bees has been estimated at 173 mg/bee/day. The current proposed risk assessment process for honey bee would have to be modified to address soil and foliar contact exposure to non-*Apis* bees. As described in the following section, such modification depends not only on the availability of data from which to estimate exposure, but also adverse effects.

5.3.3 Toxicity Testing Considerations

The main design elements of available laboratory, semi-field and open-field studies of non-*Apis* bees (*e.g.*, *Bombus spp*. and solitary bees) was recently reviewed by EFSA 2012. This review identified published laboratory test methods for:

- Bumble bees (*B. terrestris, B. impatiens*)
- Mason bees (*O. lignaria, O. bicornis)*
- Leaf-cutting bees (*M. rotundata*)
- Alkali bees (*N. melanderi*)

These test protocols mostly involved acute (<96-h) exposures to adult life stages, although some involved longer exposures to larvae during development. Exposure routes included contact, oral, residual contact and inhalation. While many of these test methods show promise for assessing risks to non-*Apis* bees, EFSA 2012 concluded that additional validation is needed prior to adoption of these test protocols into a regulatory risk assessment scheme. While many of the non-*Apis* species identified in the EFSA report are commercially available, it is uncertain whether suppliers would be able to meet year-round demand for these species should they be needed to support regulatory toxicity testing.

Semi-field protocols were reported as available for bumble bees (*B. terrestris*) and two species of solitary bees (*M. rotundata* and *O. lignaria*). As with laboratory tests of non-*Apis* bees, no formal guidelines have been established for semi-field tests with these species either. EFSA 2012 noted that protocol development for *B. terrestris* requires attention to the life cycle of the colonies (with colonies active for only 5-6 weeks) as well as nest construction in the context of evaluating effects on individual larvae. Further work on establishing reference toxicant and control acceptability criteria was also recommended to enable semi-field guidelines to be developed.

Open field study protocols were identified by EFSA 2012 for *Bombus spp.* and *M. rotundata* following similar conceptual approaches as that for *A. mellifera*. With *Bombus spp.*, however, EFSA noted that additional development is required to assess the effects of pesticides at the colony level. Due to the construction of the hive, current methods do not enable assessment of the starting condition of the colonies to be assessed. Furthermore, only an invasive approach is available for assessing effects on the numbers of brood and adults (killing the colonies at test termination). Additionally, determining appropriate study conditions for native species such as *B. terrestris* has been a challenge in designing studies (Laycock *et al.* 2012 and Whitehorn *et al.* 2012).

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5.4 Colony Models

As discussed in the problem formulation, the risk assessment process is meant to be iterative, moving from a conservative screening level, to increased levels of refinement that focus on areas where specific risks may exist. These higher tiers are intended to be increasingly representative of actual exposures and effects. The interpretation of the potential risk of pesticides on bees is complicated by several factors that are related to the nature of biology of the honey bee and of the proposed tiered system.

The Tier I method is intended to efficiently distinguish between those pesticides that do not pose a risk to bees and those pesticides where additional characterization and/or data may be required to understand the potential risks to bees. The proposed Tier I method is designed to represent upper-bound exposures to bees, and does so by estimating exposures and effects to the larvae and worker bees that receive the greatest doses of pesticides through contact or diet. Although this method is assumed to be conservative, linking potential decreases in survival of individual forager bees or larvae to the ultimate survival of the colony may be complicated due to highly dynamic and complex interactions between honey bee castes and different worker bees (*e.g.,* foragers, nurse bees, etc.). Among these complexities is the existence of multiple feedback mechanisms upon which honey bees detect and respond to natural and anthropogenic perturbations. For example, excessive loss of older forager bees can result in changes in the age-dependent polyethism (*i.e.*, task allocation among social insects) in which younger worker bees are prematurely recruited for foraging activities in order to maintain adequate resources for the colony. Although not without their own associated costs (*e.g.*, recruitment of younger foragers can lead to less successful foraging and fewer hive bees to rear developing brood), such compensatory mechanisms can temporally mask longer-term effects on the hive should effects of the stressor continue.

Although Tier II semi-field and Tier III full-field studies provide more realistic exposures as well as endpoints relevant to the entire colony, they are complicated by multiple confounding factors that are not captured by lab studies. In addition, in order to measure the impacts of a pesticide on the ultimate survival of colonies, studies require a large number of hives in order to have sufficient replication. As an alternative, many semi-field or field studies measure sublethal effects (*e.g.,* amount of brood, changes in behavior), that like with the Tier I assessment may be difficult to link to the ultimate survival of the colony. Furthermore, temporal and spatial factors such as season and availability of alternate forage

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habitat can strongly influence the colony-level response to a given stressor. These factors complicate extrapolation of adverse effects beyond the conditions associated with the study.

Given these and other challenges in the application of empirically-derived stressor-response relationships in honey bee risk assessment, it seems that at least conceptually, the use of colony-level ecological models would offer many advantages for assessing risks of pesticides. For example, by modeling the feedback loops inherent among various groups of worker bees that are differentiated by their tasks in the hive, the compensatory responses to perturbations can be explicitly assessed. In addition, pesticide effects can be considered in the context of seasonal variation in colony dynamics and spatial variation in forage habitat. Critical factors that determine colony success (*e.g.*, number of over-wintering bees and associated food reserves) can be integrated into the modeling framework and output. Importantly, such colony-level ecological models can serve as a platform for integrating the multiple measures of effects and exposure available from lower tier studies and quantitatively link these measures to endpoints that are closely aligned with the proposed assessment endpoints, which include population size and stability of managed bees and quantity and quality of hive products, such as honey.

5.4.1 Description of Desired Characteristics of Models that May be used for Risk Assessments of Bees

In order to use a colony level model for pesticide risk assessments, a desirable model would have the ability to derive measurement endpoints that are consistent with the protection goals stated in the problem formulation of this white paper. These protection goals include provision of pollination services, production of hive products (*e.g.*, honey) and contribution to pollinator biodiversity. The measurement endpoints that have been identified for use in risk assessment to meet those goals include: 1) colony strength, 2) colony survival, 3) quantity and quality of honey and 4) species richness and abundance. The first three measurement endpoints can be assessed using a colony-level model for honey bees; therefore, a desirable colony model would estimate the number of worker bees in a colony, the amount of honey the hive produces, and predict the likelihood that a colony would survive.

As discussed above, the biology of the honey bee is complex; however, in order to function, a simulation model must simplify the system which it represents. A colony-level simulation model should account for the key interactions among bees and biological characteristics (*e.g.*, diet requirements, temperature of

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the hive, unique reproductive strategy) that may impact the interpretation of pesticide effects measured as mortality to a group of bees (*e.g.*, foragers) or sublethal effects. When considering other bees or insects, the honey bee is probably the most studied. A simple search of ScienceDirect®[17] for the term "*Apis mellifera*" yields thousands of scientific articles related to various aspects of the biology of this single species. Although the biology of the honey bee is highly complex, the extent to which it has been studied suggests that realistic parameterization of a colony level model with relevant endpoints, such as queen fecundity and worker longevity, should be possible using available empirical data.

To be used for pesticide risk assessments, a model should be parameterized using exposure and toxicity data that are representative of an assessed pesticide. As discussed in the exposure characterization, dietary exposure can be estimated using Tier I methods, such as the T-REX model for foliar spray applications or the Briggs' model for soil applications. Exposures to different groups of worker bees as described by their tasks could be assessed by considering their specific dietary requirements and food consumption rates (as described in detail in Appendix I). Acute mortality data from Tier I tests or effects to brood from Tier II or Tier III studies could be incorporated into a colony level model to investigate impacts of mortality or changes in the amount of brood on the colony (as defined by survival, number of worker bees or amount of honey).

In addition, because pesticides may be applied throughout the country and throughout the year, an ideal colony model should be representative of national level usage of a pesticide (*e.g.*, application rate, frequency and timing). In cases where factors related to season or weather (*e.g.*, rainfall, temperature) impact a colony, these factors should be considered, perhaps on a regional basis. Also, the duration of the simulation should be long enough to reliably predict effects to the desired measurement endpoints.

Finally, in order to be used for risk assessment, an available model should be scientifically defensible and transparent. A desirable model would be clearly described, peer reviewed, and publically available. The sensitivity of model results to key assumptions and input parameters would also be documented along with associated uncertainties. Importantly, the results of a desirable model would be extensively evaluated using empirical results from colonies exposed to pesticides.

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5.4.2 Description of Selected Colony-level Models

A number of colony-level models with *A. mellifera* have been described in the published literature in order to address specific research objectives (e.g., investigate impacts of temperature and mites on colonies); however, a recent review of these models concluded that existing models of honey bee colonies did not seem ready for immediate use in ecological risk assessments because they do not simultaneously consider multiple stressors and link an explicit representation of foragers to the landscape, many of the models are not transparent, and sensitivity analyses and evaluations to empirically based outcomes have not been conducted for most models (Fisher and Moriarty 2011). With modifications, there are several colony-level models that have potential to be used for ecological risk assessments. Perhaps in the future, some models may be adapted for use in assessing the risks of pesticides. In order to provide background on the potential use of colony-level models to assess risks of pesticides to honey bees, a sampling of honey bee colony models obtained from the published literature are summarized below. It should be noted that this section is not intended to be comprehensive of all available colony-level models for honey bees, nor is it intended to propose and/or endorse the use of a particular model for future use.

Becher *et al.* 2010 recently developed a honey bee colony-level model to investigate the impact of brood temperature on the age at first foraging of worker bees and subsequent effects on colony dynamics. In their model, Becher *et al.* 2010 conducted a deterministic simulation of the temperature gradient across a single brood comb of unlimited size (*e.g.*, warmer areas near the center of the comb where brood were concentrated and cooler regions towards the periphery of the comb). This temperature gradient was informed by empirical measurements of hive temperature and was assumed to impact brood survival and development rate. Task-specific division of labor was addressed by considering three age classes of bees: developing brood up to 21 days old, hive bees up to 15 days old and foraging bees up to 10 days old. A maximum queen egg laying rate of 1,500 eggs/day was modified by the availability of empty cells in the comb available for depositing eggs, which in turn was determined by temperature effects on developing brood. A critical component of the model is the number of hive "heater bees" available for regulating temperature during overwintering. Under different thermal regimes, colony size was not particularly sensitive to the early onset of foraging and loss of hive bees. However, the model results indicate that colony survival depends heavily on the minimum number of bees available for heating the hive during winter. This endpoint is one of the key measures of effect

identified earlier for the design of field studies. Although informed by a number of empirical relationships, output from the model was not compared to field data on colony dynamics nor was the impact of pesticides included.

Khoury *et al.* 2011 developed a compartmental model based on simple mathematical relationships of worker bee division of labor, bee longevity and colony growth. In their model, Khoury *et al.* 2011 hypothesized that increased forager death rates would reduce social inhibition of foraging by older foragers and consequently, result in increased recruitment of younger bees into the forager workforce. This premature recruitment of foragers in turn would lead to fewer hive bees available for brood rearing and create a feedback mechanism leading to greater impacts on brood rearing. Due to the much greater mortality rate of foragers compared to in-hive bees, continued loss of foragers would result in additional demand for young foragers and subsequent reduction of hive bees. Khoury *et al.* 2011 concluded that when sustained indefinitely, a reduction in forager longevity by 2.8 days (corresponding to a death rate of 0.355 bees/day) represented a threshold for long-term colony survival. The authors caution, however, that their model was specifically developed to investigate how changes in forager mortality rate affected division of labor and colony growth and not intended for application beyond these questions.

Schmickl and Crailsheim 2007 developed one of the more complex models of honey bee colony dynamics called "HoPoMo." This model considers many factors that influence colony size dynamics including: seasonal variation in queen egg laying rate, availability of pollen stores, temporal polyethism among worker bees in relation to division of labor, three stages of brood development (egg, larvae, and pupae), larval cannibalism by workers, availability of empty comb for egg laying, efficiency of brood nursing as influenced by the ratio of larvae to nurse bee, regulation of foraging demand for nectar and pollen resources, influence of weather conditions (temperature and precipitation)and even swarming activity. Several of the model algorithms were verified with empirical measurements from previous studies (*e.g.*, overall worker bee life expectancy, seasonal growth of adult bees and brood cells, growth of pollen stores and overall colony weight). A sensitivity analysis indicated that mortality rates were among the most influential set of input parameters. Due to its complexity, HoPoMo uses 60 differential equations in order to describe the multiple feedback loops and hive dynamics. While the overall pattern of colony dynamics simulated by HoPoMo appears to agree with empirical measurements of colony strength, application of the model for evaluating the effects of pesticide exposure was not evaluated at the time of its publication. Thus, the extent to which HoPoMo would accommodate laboratory and field-

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derived exposure and effects data without significant modification is unclear.

DeGrandi-Hoffman *et al.* 1989 published a model called "BeePop," which models the dependency of queen egg-laying rate on the size of the adult population, temperature and photo-period. The task division among bee castes is represented by a fixed age of bees and is not altered by outside perturbations. The model consists of two major components, *i.e.*, EGGLAY and COLPOP; EGGLAY determines the number of eggs laid by the queen at any time (*t*) and the percentage of eggs that will develop into workers and drones is assigned in a subroutine. COLPOP tracks the development of eggs to adults and predicts the colony's adult population size at any time (*t*). The BEEPOP model estimates the number of eggs laid by the queen per day at any time (*t*) as a function of ambient temperature, photoperiod and adult population size. The proportion of eggs developing into drones is determined by the amount of sperm remaining in the queen's spermatheca (which is a function of the age of the queen and the number of drones with which she mated) as well the photoperiod and the foraging population size in the colony. The model then estimates the number of larvae that develop into adults (assuming that only 85% emerge as adults) and the number of adults which become foragers in 21 days after emergence. The user specifies the number of days which forager bees live and the model estimates the colony size in terms of total number of adult bees.

Thompson *et al.* 2005 is one of the few publications to model the impacts of pesticides on colony dynamics that adapted the model of *Varroa* mite (*Varroa spp.*) infestation growth by Wilkinson and Smith 2002 to predict the impacts of four pesticides on colony size. In their model, the authors considered the impact of pesticides including an insect growth regulator, chiton synthesis inhibitor, ecdysteroid synthesis inhibitor, and an ecdysteroid analogue on queen egg laying rate, brood development and two castes of adult workers (*i.e.*, younger nurse bees and older forager bees). Seasonal aspects of queen egg laying and adult mortality rates were incorporated into model predictions. In addition to direct effects on bee survival, indirect effects were simulated based on reduced availability of nurse bees for rearing brood via premature recruitment of foragers and increased mortality rates of foragers. Importantly, the authors demonstrated the different effects of forager and brood mortality which occurred at different times of the year. The primary output of the model was the size of the colony available at over wintering which is considered an important indicator of hive survival during the following spring.

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Makela *et al.* 1993 developed a model that included colony-level and *Apis* population level dynamics that could be applied spatially to a landscape to predict the spread of African honey bees. This model accounted for intra-colonial parameters, including ages of worker and queen bees, numbers of eggs laid, amounts of food stored in the hive as well as genetics as they could distinguish between a European and Africanized hive. The model distinguished between different types of worker bees (according to their job). Colony-level outputs included several that are relevant to the defined protection goals, including number of worker bees, amount of brood, mass of honey and pollen stored in the hive and age of worker bees. The model also predicted population-level outcomes, including the spatial distribution of colonies over time and the genetic make-up of the colony (*i.e.,* the extent to which a colony was Africanized). The model was not evaluated using empirical results relevant to the colony-level and population-level predictions. Also, this model does not consider impacts of pesticide exposures on the intra-colonial parameters. Despite those limitations, this model presents an interesting example of how a simulation model can generate spatially explicit, population-level outcomes that reflect colony dynamics and the reproduction of bees.

5.4.3 Summary Regarding Colony-level Models

Although current models of honey bee colonies appear to require additional development and evaluation prior to their immediate use in ecological risk assessments for pesticides, a number of the available colony-level models (or portions of them) have potential to be used for ecological risk assessments. At this time, EPA is considering the potential utility of these models in integrating diverse types of information related to pesticide exposures and effects in the context of the complex biology of a honey bee colony and the desired protection goals. The discussion above focused on some considerations that may determine the utility of a model for ecological risk assessments followed by a description of a subset of models that are available in the published literature. The EPA is interested in the SAP's thoughts on the potential utility of colony level models, including future considerations for developing these types of models into useful tools for pesticide risk assessment purposes.

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6 Epilogue

This white paper has provided a description of the proposed process depicted earlier in **Figures 2** and **3** for evaluating the potential risks of pesticides to honey bees. The proposed process is relatively consistent with the process used to evaluate risk to other taxa in that it is both tiered and iterative and it makes use of similar models for estimating exposure. At Tier I, the processes serves as a screen that is based on relatively conservative estimates of exposure that reduce the likelihood of making a Type II error, *i.e.*, concluding that there is not an effect when there is, while attempting to minimize Type I errors, *i.e.*, concluding that there is an effect when there is not. Achieving this balance can be challenging since the Tier I screen is based on effects to individual bees in a laboratory while the actual effect of interest is whether there are adverse effects to the colony as a whole. A screen that is too conservative/protective will not serve as an effective screen but rather as a wall requiring that all chemicals undergo higher-tier exposure and/or effect testing. This would result in an unreasonable level of resources both on the part of the agencies tasked with reviewing such data as well as on the regulated community tasked with conducting such studies. Although the process is characterized as quantitative, deterministic RQ values are only calculated at a screening level while Tier II and Tier III assessments are intended to be qualitative and will rely on best professional judgment regarding the integration of a wide range of data rather than an LOC. Measured residue values from Tier II and III exposure studies are proposed for use in refining RQ values in the screening-level assessment process; however, colony-level effects studies at Tier II and III are not proposed for use in refining RQ values. The proposed process provides information on exposure and effects for both individual bees and at the colony level; however, there is uncertainty as to the extent that the process can be considered representative of native species. In the absence of an alternative approach for the non-*Apis* bees, the proposed process for honey bees may represent the best available science at this time. The science underlying the proposed process has evolved considerably over the past 10 years and it is likely that it will continue to evolve and become more inclusive of non-*Apis* species. Once exposure and effect studies are available for native species, the regulatory process will likely accommodate these changes particularly if it becomes apparent that the current process for evaluating risk is inadequate.

The use of colony-level modeling is expected to enable risk assessors to estimate potential effects on honey bee colonies using lower-tier effect and exposure estimates and will better enable the results from multiple lines of evidence to be integrated. Once sufficiently developed and vetted, the models can

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be used to characterize the likelihood that a colony may not survive given Tier I RQ values that exceed the LOC. These models could identify which endpoints should be the focus of Tier II semi-field studies and where both Tier I and Tier II data are available, what is the likelihood of colony loss. Such modeling efforts could help identify remaining uncertainties that should be the focus of Tier III studies if required to support risk management decisions.

Significant challenges remain in interpreting the results of full field studies. For example, **Figures 12** and **13** depict the pattern of honey bee mortality (mean number of dead adults, larvae and pupae combined) and overall colony strength (in mean number of bees per hive), respectively, following the planting of treated seed. **Figure 12** depicts a series of spikes in mortality; however, the majority of the peak mortality events were associated with periods when the colonies are being assessed (opened) either by the beekeeper or the researchers. This represents an important consideration in terms of how much colonies have to be manipulated in order to collect measurement endpoints and how these measurements impact the proper maintenance and function of the colony. Although there are multiple peaks in mortality throughout the course of the study, the highest levels of mean mortality occurred prior to initiation of the study in both treated and control colonies. While treated colonies appear to exhibit higher levels of mortality during each peak mortality event, it is uncertain whether the average level of mortality is having a detrimental effect on the colonies. **Figure 13** depicting colony strength suggests roughly a two week period when treated colonies exhibited fewer bees relative to control colonies; however, afterwards there was no apparent difference between controls and treated colonies based on this measurement endpoint. Although it is difficult to interpret the overall outcome of this study by examining these two measurement endpoints alone, the data underscore the challenges in understanding the extent to which a particular parameter would have to change to have a meaningful impact at the colony level. While there are transient effects during the study, these effects (taking the graphs at face value) do not appear to have affected the overwintering success of the colonies.

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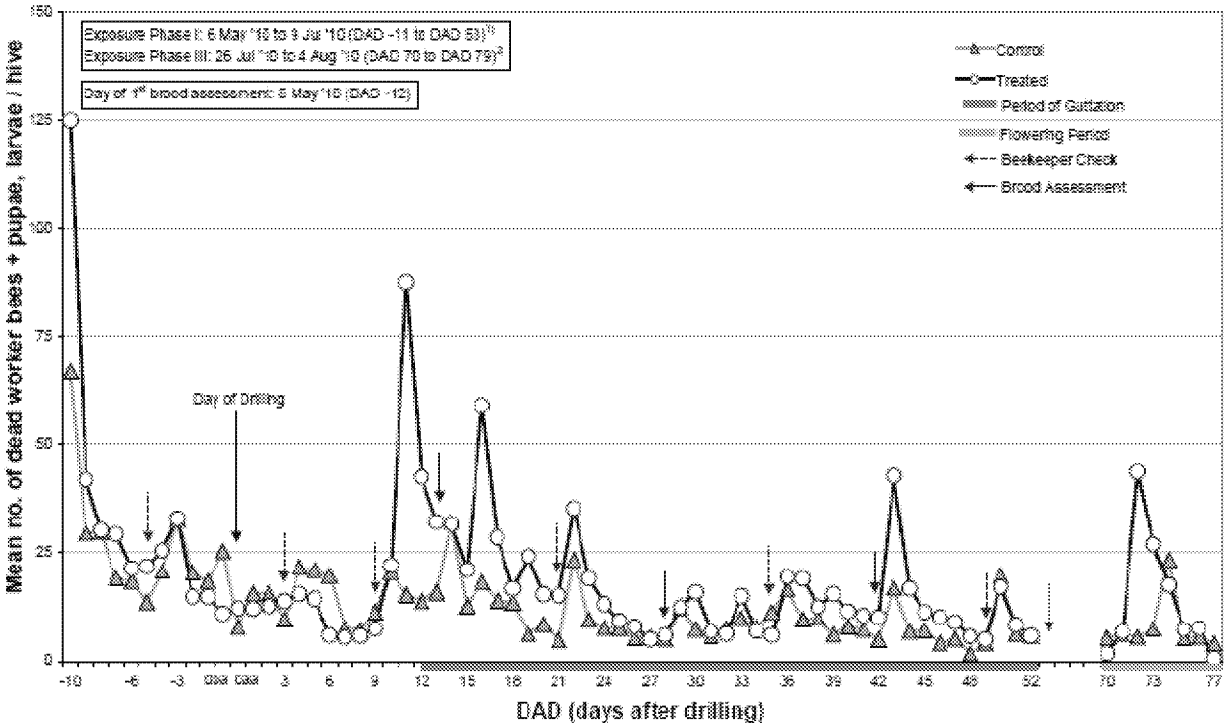

Figure 12. Adult honey bee mortality following planting of treated seed.

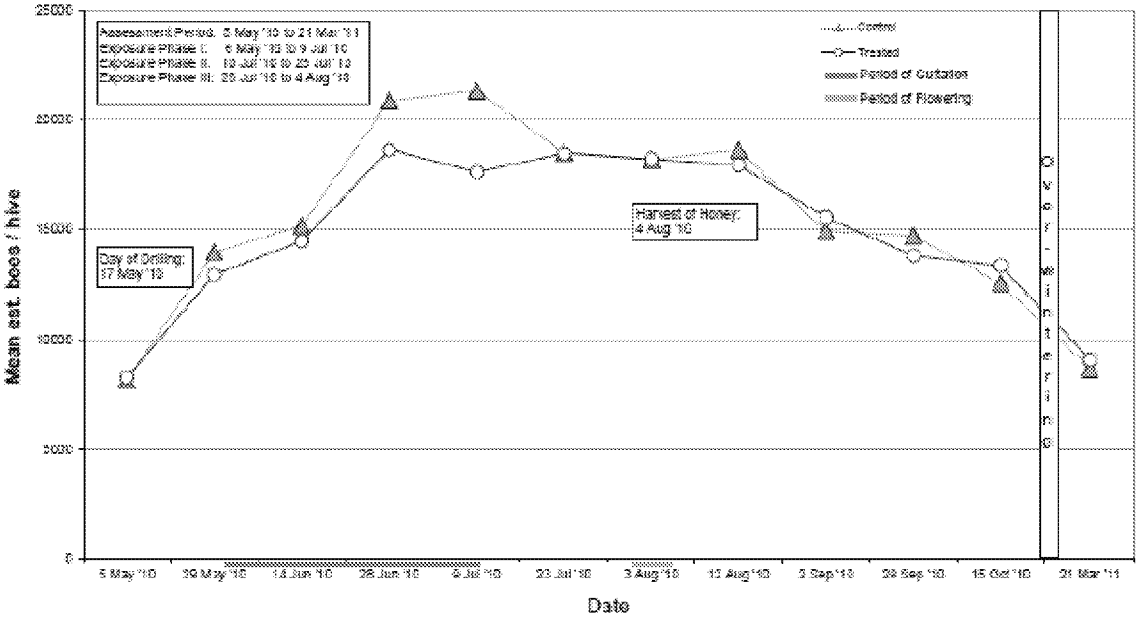

Figure 13. Overall honey bee colony strength (mean number of bees per hive) following planting of treated seed.

As indicated in this paper, the science of evaluating the potential effects of pesticides on honey bees and more broadly on non-*Apis* bees, continues to evolve. It is important that sufficient guidance is developed to assist in the uniform conduct and interpretation of studies; however, as studies become more environmentally relevant, the ability to maintain uniformity is reduced. With increased realism, the field studies are subject to a number of confounding effects that have already been discussed; however, to the extent that the Tier III studies can be contoured to address specific uncertainties identified in lower tiers and to the extent that the results of these studies are considered in the context of lower-tier studies and other scientifically relevant information, risk assessors will be able to utilize a weight-of-evidence approach to develop consistent and plausible risk assessments for bees.

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Appendix 1. Estimation of food consumption rates for worker larvae and adults

As discussed in the effects characterization, acute oral toxicity data are necessary for adult and larvae in order to characterize the risks of a pesticide. Because these toxicity data are expressed on a dose basis (*i.e.,* μg a.i./bee), it is necessary to convert estimated concentrations of pesticides in bee food (expressed as mg a.i./kg) into doses. Honey bees fulfill their nutritional requirements through consumption of nectar, honey, pollen and bee bread. In addition, bees require royal jelly and brood food (also referred to as brood jelly) which are composed primarily of glandular secretions produced by nurse bees, mixed with some honey. In the proposed approach, pesticide doses received by bees can be calculated using nectar and pollen consumption rates for larval and adult worker bees. In this approach, it is assumed that exposures through consumption of nectar and pollen are conservative representations of potential exposures through consumption of honey and bee bread, respectively. This approach is likely to be conservative because it assumes that pesticides do not degrade while honey and bee bread are stored in the hive. For bees that consume honey, it is assumed that the estimated pesticide exposures can be related back to the original concentration in nectar by accounting for the amount of sugar consumed by bees. It is also assumed that pollen and nectar consumption rates and resulting exposures are protective of exposures of bees to pesticides through consumption of royal jelly and brood food, which are expected to have lower concentrations of pesticides compared to pollen and nectar.

Because the diet of adult worker bees changes in content and amount as they age and their tasks evolve, the doses of adult bees are expected to vary. For the Tier I risk assessment method, which is intended to generate conservative, exposure estimates are intended to be based on food consumption rates for the age/task of bees expected to receive the greatest exposures. This appendix discusses pollen and nectar consumption rates for different types of bees, building upon work published by Rortais *et al.* (2005), Crailshaim *et al.* (1992 and 1993) and others. Food consumption rates are proposed for larvae and the most susceptible adult worker bees. In addition, comparisons are made between the proposed food consumption rates and other bee castes (*i.e.,* drones and queens).

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Food consumption of larval worker bees

The average duration of the larval life stage of honey bee workers is 5-6 days (Winston 1987). Over the course of the life stage, larvae consume different types and amounts of food. During the first three days, larvae consume a total of 30 mg (Nelson 1924 as described by Rortais *et al.* 2005) of royal jelly and brood food, both of which are produced by adult nurse bees (Winston 1987). The daily food consumption rate during the first three days of the larval stage is not likely to be equivalent from one day to the next since larvae are growing exponentially. Therefore, if the food intake rate is also exponential, approximate food consumption rates of larvae would be 3.75, 7.5 and 15 mg on days 1, 2 and 3, respectively. During the fourth and fifth days of the larval life stage, larvae are fed bee bread (or pollen) and honey (or nectar). The nectar and pollen consumption rates of larvae during days 4 and 5 of the uncapped phase are discussed below.

Nectar (and honey)

Rortais *et al.* 2005 based their estimated sugar consumption rates of larvae (age 4 and 5 days), on data published by Bishop 1961) indicating that they consume 120 mg food over this two day period. If it is assumed that 5.4 mg of this is represented by pollen (see next section), then it can be assumed that larvae consume 115 mg honey during this time period. As discussed above, the growth rate of larvae is exponential; therefore, the honey consumption rate of larvae is expected to increase exponentially each day to support that growth rate. Based on that assumption, the honey consumption rate for days 4 and 5 of the larval period can be estimated as 37 and 78 mg. If it is assumed that larvae are fed honey diluted with water so that the food of larvae is 45% sugar (Winston 1987, Rortais *et al.* 2005) and nectar contains an average of 30% sugar (See Table 1-4), this is equivalent to consumption of 56 and 117 mg of nectar for days 4 and 5, respectively. The estimated nectar consumption rate of 5 day old larvae is greater than the amount of royal jelly and brood food that they consume during the entire first three days of the larval life stage, which suggests that the use of nectar consumption rates of 5 day old larvae are protective of consumption rates of royal jelly and brood food.

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Pollen (and bee bread)

Rortais *et al*. 2005 suggest that worker larvae consume 5.4 mg pollen during larval development. This value is based on data reported by Simpson 1955 that corresponded to consumption of red clover pollen. Simpson 1955 observed a large amount of variability in the amount of pollen consumed by larvae, with some larvae consuming more than 5.4 mg pollen and some larvae consuming none. Babendreier *et al*. 2004 reported that larvae consumed 1.5-2 mg of maize pollen during the larval development stage, noting significant differences in the masses of pollen consumed by larvae from different colonies. Since larvae only consume pollen during the last two days of this life stage (Winston 1987), available information indicate that pollen consumption rates are 0.75 to 2.7 mg (**Table 1-1**).

Table 1-1. Daily pollen consumption rates of larval worker bees during the last two days of the larval life stage.

Source	Pollen consumption rate (mg/day)*	Pollen species
Simpson (1955)	2.7	Red clover
Babendreier *et al*. (2004)	0.75-1	corn

*Calculated by dividing amount of pollen consumed over entire life stage by 2 days.

Proposed food consumption rate for larval worker bees

As described in the exposure section of this white paper, for each type of application (*i.e.*, foliar spray, soil treatment, seed treatment and tree injection/drench), a pesticide residue concentration in one plant matrix (plant leaves) is proposed to represent residues in both pollen and in nectar. Effectively, the proposed Tier I methods for these types of application assumes that pesticide concentrations in pollen and in nectar are equivalent. Thus, the Tier I estimated daily dose received by larvae can be calculated by multiplying the pesticide concentration by the food consumption rate of larvae as represented by the sum of daily pollen and nectar consumption (*i.e.*, 2.7 and 117 mg/day, respectively). Therefore, the proposed food consumption rate of larvae is 120 mg/day.

Discussion

The purpose of this analysis is to derive food consumption rates that can be related to pesticide concentrations in food sources collected by bees (*i.e.,* nectar and pollen). On the surface, the proposed food consumption rate of 120 mg/day for 5 day old larvae appears to be equivalent to the food consumption rate larvae over days 4 and 5 combined (as suggested by Rortais et al. 2005); however, the

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proposed value is different because it is adjusted to represent consumption of the original source material (*i.e.,* nectar) for the honey that the larvae are actually eating. As discussed above, the adjustments are made by assuming that the sugar contents and pesticide concentrations in the food consumed by the larvae are constant for honey and nectar and only the water contents change.

Although exposures through consumption of royal jelly and brood food are not explicitly accounted for in the proposed Tier I exposure method, the proposed method is expected to be conservative for two primary reasons. First, because the daily food consumption rate proposed for larvae is much larger than the consumption rate of brood food, which is 30 mg over three days. Second, because pesticide concentrations in pollen and nectar are expected to be greater than those in royal jelly and brood food. This is supported by work by Davis and Shuel 1988 and Kamel et al. (unpublished) that demonstrated that pesticide concentrations in food consumed by nurse bees were 2-4 orders of magnitude higher than concentrations measured in royal jelly. Davis and Shuel 1988 dosed nurse bees with 13 ppb carbofuran or 12 ppb dimethoate, and measured concentrations of 0.0020 ppb carbofuran and 0.0029 ppb dimethoate in royal jelly of exposed nurse bees. Kamel *et al.* (unpublished) exposed nurse bees to 100 ppb imidacloprid in pollen and measured imidacloprid concentrations of 0.27-1.02 ppb in royal jelly. Although royal jelly differs in composition from brood food, both consist of secretions from the hypopharyngeal and mandibular glands of nurse bees. Therefore, the observations that pesticide concentrations in royal jelly may be orders of magnitude lower than the diet of the nurse bees, apply to the food consumed by larvae during the first 3 days of the uncapped life stage.

Food consumption of adult worker bees

Adult worker bees consume nectar, honey, pollen and bee bread. Over the course of the adult life stage, an adult worker bee will require different portions of these food items in order to meet the demands of the different tasks a worker must fulfill. For example, as the metabolic requirements of a bee increase, for example while foraging , the bee will require more nectar (or honey) to meet the energy demands of flying. Also, bees that produce brood food and royal jelly (*i.e.*, nurse bees) require the most pollen (compared to other bees engaged in different tasks) because their glandular secretions provide protein and lipids to larvae and the queen (Winston 1987).

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Nectar

Nectar consumption rates for different types of worker bees were calculated by Rortais *et al.* (2005) (**Table 1-2**). The estimated nectar consumption rate of nectar foraging bees (*i.e.*, 107-428 mg/day) is of interest for the Tier I exposure assessment method because it represents the highest estimated nectar consumption rate of adult worker bees. This suggests that nectar foraging bees would be potentially receive the highest pesticide exposures through consumption of contaminated nectar.

Table 1-2. Estimated nectar consumption rates of adult worker bees by task and approximate age.

Task*	Rortais *et al.* (2005) task description	Average age (in days)*	Nectar consumption rate (mg/day)**
Cell cleaning and capping	Wax producer	0-10	60
Brood and queen tending (nurse bees)	Brood attending	6-17	113-167
Comb building, cleaning and food handling	Wax producer	11-18	60
Foraging (pollen)	Forager	>18	35-52
Foraging (nectar)	Forager	>18	107-428

*From Winston 1987

**Adapted from Rortais *et al.* 2005 using sugar consumption rates and assuming that the sugar content of nectar is 30% (average of data in Table 1-4).

The nectar consumption rate of nectar foraging worker bees is based on **Equation 1-1**. Because there are 5 different variables that influence the calculation of the nectar consumption rate for nectar foragers, there is potentially a great deal of variability that influences this estimate. In addition, this approach is limited in that it does not account for the energy (*i.e.,* sugar) requirements of bees while they are at rest.

Equation 1-1 $D_{nectar} = \frac{T*S_F*D*F}{P}$

Where (values used by Rortais *et al.* 2005 in parentheses):

S_F is the amount of sugar required for flight (8-12 mg/hr)
T is the average number of foraging trips made per day (10)
D is the duration of each foraging trip (0.5-1.33 hr)
F is the fraction of the foraging trip spent flying (0.8)
P is the amount of sugar present in nectar (1 mg sugar per 2.5 mg nectar or 40% sugar)

An analysis was conducted by EPA to explore the ranges of nectar consumption rates when the variability in parameter values is considered along with the influence of the resting metabolic requirements of bees. This analysis was based on a Monte Carlo simulation (conducted using Crystal Ball 2000), where 10,000 nectar forager honey bees were simulated using randomly selected values from

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distributions of the 5 variables identified above. In addition, this approach accounted for account for the energy requirements of bees while they are at rest.

For this simulation, the nectar consumption rate of an individual bee was calculated using **Equation 1-2**, which represents a modification to the approach used by Rortais *et al.* (2005). The approach depicted by this equation is as follows. For each simulated bee, the number of trips (T) made in one day was randomly selected. Then, for each trip, the duration (D_i), fraction of each trip spent flying (F_i) and the sugar content of the collected nectar (P_i) was randomly selected. The assumptions associated with each of the variables are provided in **Table 1-3**. This approach assumes that the bees will forage at different locations throughout the day. The amount of sugar required for each trip (to meet the energetic demands of flying) was calculated by multiplying the duration of the individual foraging trip by the fraction of the trip spent flying by the bee's sugar requirement for flying. The total amount of sugar required for flying over the entire day was calculated by adding up the amount of sugar required for all of the day's trips. In addition, the amount of nectar required to meet the bee's energy demands while resting during the day was calculated by multiplying the amount of time in the day when the bee was not flying by the amount of sugar required per hour to meet the bee's resting metabolic rate (S_R). A value of 0.7 mg/hour was selected based on Winston (1987). This value was converted to the amount of nectar required to meet the bee's resting metabolic rate by dividing S_R by the average sugar content of nectar from various plants (*i.e.,* 30%,**Table 1-4**). Based on this analysis, the median and 95th percentile estimates of total nectar consumption by nectar forager bees are 292 and 499 mg/day, respectively (**Table 1-5**). The median estimate of 292 mg/day is proposed for use in estimating pesticide doses to honey bees.

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Equation 1-2 $D_{nectar} = S_F * \sum_{i=0}^{T} \left(\frac{D_i * F_i}{P_i}\right) + \frac{S_R * [24 - (\sum_{i=0}^{T} D_i * F_i)]}{P_{ave}}$

Where:

S_F is the amount of sugar required for flight
S_R is the amount of sugar required to meet resting metabolic rate
F_i is the fraction of time spent flying during trip *i*
D_i is the duration of foraging trip *i*
P_i is the amount (mg) of sugar in nectar collected during trip *i*
P_{ave} is the average sugar content in nectar

Table 1-3. Parameter assumptions used in Monte Carlo analysis of nectar consumption rates of nectar foraging bees.

Variable	Distribution assumption *	Mean	Standard Deviation	Minimum	Maximum	Source(s)
Number of trips per day	Lognormal	10	3	1	150	Winston 1987
Sugar requirement during flight (mg/hr)	uniform	NA	NA	7	12	Balderrama *et al.* 1992; Gmeinbauer and Crailsheim 1993
Duration of single foraging trip (hr)	uniform	NA	NA	0.5	1.33	Winston 1987
Fraction of trip spent flying	uniform	NA	NA	0.5	0.9	It is assumed that the bee will spend a portion of the foraging trip flying and the rest of the time collecting nectar. Rortais *et al.* 2005 assumed a value of 0.8.
Sugar content of nectar	lognormal	0.3	0.1	0.1	0.6	**Table 1-4**

NA = not applicable
*In cases were no information were available to inform the distribution shape assumption, a uniform distribution was selected.

Table 1-4. Sugar content in nectar of different plants.

Plant species (common name) *	% sugar in nectar	Source
Raphanus raphanistrum (charlock)	25-58	Butler 1944
Malus domestica (Apple)	32-56	Butler 1944**
Tilia platyphyllos (lime)	33-55	Butler 1944
Taraxacum dens-leonis (Dandelion)	15-55	Butler 1944
Prunus cerasus (wild cherry)	21-52	Butler 1944
*Cucurbita pepo (*Pumpkin)	40.5-50.5	Das Graças Vidal *et al*. 2006
Helianthus annuus (sunflower)	40	Pham Delègue and Bonjean (1983) as cited by Rortais *et al.* (2005)
Brassica sp. (savoy cabbage)	39	Butler 1944
Rubus idaeus (raspberry)	19-39	Butler 1944
Prunus sp. (plum)	7-38	Butler 1944
Trifolium repens (wild white clover)	12-36	Butler 1944
Trifolium repens (white clover)	32.4	Wykes 1952
Tilia x vulgaris (common lime)	32.3	Wykes 1952
Rubus fruticosus (blackberry)	11-31	Butler 1944
Onobrychis viciaefolia (sainfoin)	17-30	Butler 1944
Rubus loganobaccus (loganberry)	30.0	Wykes 1952
Lavandula spica (lavender)	28.2	Wykes 1952
Chamaenerion angustifolium (rosebay willow-herb)	26.2	Wykes 1952
Borago officinalis (borage)	26.1	Wykes 1952
Lotus corniculatus (bird's foot trefoil)	26	Butler 1944
Rubus fruticosus (blackberry)	25.7	Wykes 1952
Ribes nigrum (black currant)	25	Butler 1944
Crataegus oxyacantha (hawthorn)	2-22	Butler 1944
Pycnanthemum pilosum (mountain mint)	19.8	Wykes 1952
Trifolium pratense (red clover)	17.9	Wykes 1952
Pyrus sp. (Pear)	15	Butler 1944
Sinapis alba (white mustard)	13.9	Wykes 1952
Hedera helix (ivy)	11.0	Wykes 1952

*The average sugar content in nectar from these genera (multiple values for the same genus are averaged) is approximately 30% (stdev = 10%).
**Butler observed that sugar content for the same type of plant varied among individuals and among samples collected from the same individual.

Table 1-5. Results of Monte Carlo analysis for nectar consumption rates of nectar foraging bees.

Percentile	Time spent (hr)		Amount of nectar consumed (mg)		
	Flying	Resting	While flying	While resting	Over entire day
5%	4.9	19.1	128	44	173
10%	6.3	17.7	150	41	192
25%	8.3	15.7	195	37	232
50%	8.9	15.1	256	35	292
75%	9.9	14.1	333	33	366
90%	8.7	15.3	415	36	451
95%	11.8	12.2	471	28	499

Pollen (and bee bread)

Crailsheim *et al*. 1992 measured pollen contents in gastrointestinal tracts (including crops, midguts and recta) of adult worker bees aged 1, 4, 9, 16, 23 and 30 days. These were used to represent the daily pollen consumption rates of individual bees. Consumption rates of the different ages and their corresponding tasks (based on Winston 1987) are provided in **Table 1-6.** Based on this information, young bees and nurse bees consume the largest amounts of pollen. According to Crailsheim *et al*. 1992, forager bees consume a "negligible" amount of pollen.

Table 1-6. Pollen consumption rates of adult worker bees by task and approximate age.

Task*	Average age of task (days)*	Age where pollen consumption was reported (days)**	Pollen consumption rate (mg/day)**
Cell cleaning and capping	0-10	1	2.2
		4	8.2***
Brood and queen tending (nurse bees)	6-17	9	9.5
		16	1.7
Comb building, cleaning and food handling	11-18	16	1.7
Foraging	>18	23, 30	0.041

*From Winston 1987
**Estimated from Table 4 of Crailsheim et al. (1992). Consistent with Table 1 of Szolderits and Crailsheim 1993.
***This may also include nurse bees.

Proposed food consumption rate for adult worker bees

As discussed above, the proposed Tier I methods for estimating dietary exposure assume that pesticide concentrations in pollen and in nectar are equivalent. Thus, the most susceptible group of adult worker bees, as defined by task, can be identified by considering the total amount of pollen and nectar consumed by the different groups of bees. When nectar and pollen consumption rates are considered for the different tasks of worker bees, nectar foraging bees have the highest food consumption rates among adult worker bees (**Figure 1-1**). Based on this information, the food consumption rate of 292 mg/bee is proposed for incorporation into the Tier I exposure method to derive dose-based estimates of exposure to adult worker bees consuming pollen and nectar.

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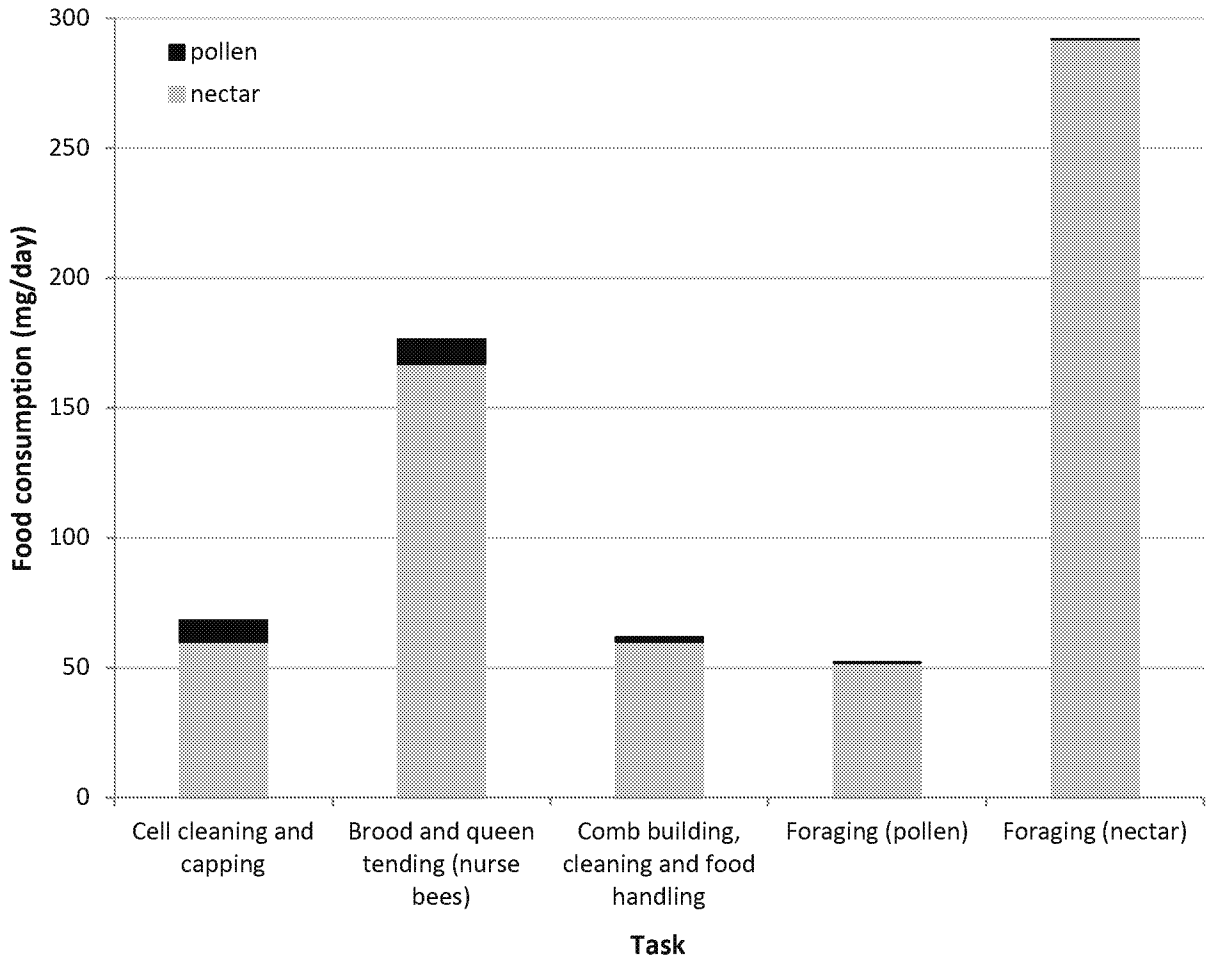

Figure 1-1. Food consumption rates of adult worker bees by task.

Discussion

The proposed food consumption rate for adult worker bees is based on the median estimate of nectar consumption of nectar foraging bees. The median value is selected because it is considered most likely to represent food consumption of this group of bees. In addition, use of the median value avoids compounding conservative assumptions in the overall tier I exposure method. For example, if an upper bound food consumption rate for nectar foragers was proposed, the overall exposure value would most likely exceed the desired 95th percentile for adult worker bees due to the combination of an upper bound estimate of residues in pollen and nectar with an upper-bound estimate of consumption rates.

This method described above focuses on exposures to honey bees using food consumption rates that correspond to summer bees. Winter bees may also be exposed to pesticides through consumption of

food that is stored in the hive. Rortais *et al.* 2005 estimated sugar consumption rates of 8.8 mg sugar/day for winter bees. Although these bees are consuming sugar through honey, this value can be correlated to a concentration in the nectar used to create the honey by considering the sugar content of nectar (average value of 30%). This yields a relative nectar consumption rate of 29 mg nectar/day, which is an order of magnitude below the proposed food consumption rate for adult worker bees (*i.e.,* 292 mg/day). Crailsheim *et al.* 1993 compared pollen consumption of winter and summer bees. When compared to summer nurse bees, which have the highest pollen consumption rates among summer nurse bees, winter bees consumed an order of magnitude less pollen on a daily basis, which is higher than the pollen consumption rate of nectar foraging bees. Despite that, the total food consumption rate of nectar foraging bees is protective of daily estimated exposures potentially received by winter bees consuming food stored in the hive.

The Tier I exposure method proposes to use a total food consumption rate to estimate dietary exposures to larvae and adult bees because the Tier I methods assume that pesticide concentrations in pollen and nectar are equivalent (*i.e.,* there is effectively one EEC for bee food). On a case-by case basis, the risk assessor may choose to use alternative food consumption rates or to separate out consumption rates for pollen and nectar. If data are available where the assessed pesticide is measured in pollen and in nectar and the concentration in pollen is much higher than nectar, the risk assessor may choose to break out the consumption rates. In that case, nurse bees may be at a greater risk than the nectar foragers. In addition, there may be interest in estimating exposures to different groups of worker bees for colony-level modeling. This can be accomplished using food consumption rates for different worker bees or castes. The latter is discussed in the following section. **Table 1-7** summarizes the food consumption rates for larvae and adult worker bees (described above) and drones and queens (described below).

Comparison of proposed consumption rates and resulting exposures to other castes

Although the focus on the current Tier I exposure method is on worker bees, it is also expected to generate estimates of exposure that are protective for drones and queens. **Table 1-8** provides an illustration of the relative exposures of larvae of different castes and different adult worker bees by tasks and drones. Pesticide doses in this table are estimated using food consumption rates included in **Table 1-7**. To illustrate the relative doses potentially received by bees consuming food with the same

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level of pesticide contamination, doses are calculated for nectar and pollen that contain concentrations of 100 µg a.i./kg. It is assumed that the concentration in brood food and royal jelly is 1 µg a.i./kg (*i.e.*, 100x lower than pollen and nectar) based on Davis and Shuel 1988 and Kamel *et al.* (unpublished). The results of this analysis are discussed below.

Table 1-7. Estimated food consumption rates of bees.

Life Stage	Caste (task in hive*)	Average age (in days)*	Daily consumption rate (mg/day)			
			Brood food / royal jelly	Nectar**	Pollen***	Total food
Larval	Worker	1	3.75	none	none	3.75
		2	7.5	none	none	7.5
		3	15	none	none	15
		4	none	37	2.7	40
		5	none	117	2.7	**120**
	Drone	5	none	52	unknown	>52
		6	none	100	unknown	>100
	Queen	1	9.4	none	none	9.4
		2	19	none	none	19
		3	38	none	none	38
		4	100	none	none	100
		5	203	none	none	203
Adult	Worker (cell cleaning and capping)	0-10	none	60	2.2-8.2	47-53
	Worker (brood and queen tending, nurse bees)	6-17	none	113-167	8.2-9.5	93-135
	Worker (comb building, cleaning and food handling)	11-18	none	60	1.7	47
	Worker (foraging for pollen)	>18	none	35-52	0.041	26-39
	Worker (foraging for nectar)	>18	none	292+	0.041	**292**
	Worker (maintenance of hive in winter	0-90	none	29	2	31
	Drone	>10	none	133-337+	0.0002	133-337
	Queen	0+	Unknown	unknown	None	unknown

NA = not applicable
*From Winston 1987
**From Rortais *et al.* 2005. Assumes that average sugar content of nectar is 30%.
*** From Crailsheim et al. (1992, 1993).
+Calculated by USEPA, as described in this appendix.

Table 1-8. Pesticide doses received by different bees based on estimated food consumption rates of bees (from Table 1-7), assumption that pesticide concentration in pollen in nectar is 100 µg a.i./kg and that concentration in brood food and royal jelly is 1 µg a.i./kg (*i.e.*, 100x lower than pollen and nectar).

Life Stage	Caste (task in hive*)	Average age (in days)*	Daily dose (µg a.i./bee per day)			
			Brood food / royal jelly	Nectar**	Pollen***	Total food
Larval	Worker	1	0.00375	0	0	0.00375
		2	0.0075	0	0	0.0075
		3	0.015	0	0	0.015
		4	0	3.7	0.7	4.0
		5	0	11.7	0.27	**12**
	Drone	5	0	52	unknown	>5.2
		6	0	100	unknown	>10
	Queen	1	0.0094	0	0	0.0094
		2	0.019	0	0	0.019
		3	0.038	0	0	0.038
		4	0.100	0	0	0.100
		5	0.203	0	0	0.203
Adult	Worker (cell cleaning and capping)	0-10	0	4.5	0.22-0.82	4.7-5.3
	Worker (brood and queen tending, nurse bees)	6-17	0	8.5-12.5	0.82-0.95	9.3-13.5
	Worker (comb building, cleaning and food handling)	11-18	0	4.5	0.17	4.7
	Worker (foraging for pollen)	>18	0	2.6-3.9	0.0041	2.6-3.9
	Worker (foraging for nectar)	>18	0	29.2	0.0041	**29.2**
	Worker (maintenance of hive in winter	0-90	0	2.9	0.2	3.1
	Drone	>10	0	13.3-33.7	0.00002	13.3-33.7
	Queen	0+	unknown	unknown	none	unknown

Drones

According to Rortais *et al.* 2005, drone larvae consume 46.2 mg sugar during the last two days of the larval life stage (*i.e.,* days 5 and 6). If it is assumed that drone larvae grow exponentially and that their food consumption rates increase each day, then estimated sugar consumption during days 5 and 6 of the larval period would be 15.5 and 30.7 mg, respectively. If it is assumed that this sugar originates from honey where the original nectar source had 30% sugar (based on the average concentration of sugar in nectar from **Table 1-4** this is equivalent to 52 and 100 mg nectar on days 5 and 6, respectively. The highest of these values is similar to the nectar consumption rate for workers (*i.e.,* 117 mg/day). A literature search conducted in July 2012 by EPA was unsuccessful at locating pollen consumption data for drone larvae. Despite the lack of pollen consumption data for drone larvae, if the pollen consumption rate for drones is on the same order of magnitude as workers, the proposed food consumption rate based on larval workers is expected to be protective for drone larvae.

Adult drones consume honey and pollen. Drones spend the majority of their time resting in the hive, with younger drones (8 days old) rarely leaving the hive except perhaps for orientation flights that last only a few minutes. Older drones make an average of 3-5 mating flights that last 25-32 minutes. The energy requirements of adult drones are 1-3 mg sugar/hr while resting and 14 mg sugar/hour while flying (Winston 1987). Therefore, the food requirements and potential dietary pesticide exposures of older drones that make mating flights are expected to be greater than younger adult drones. When considering the amount of time flying per day and the amount of time resting, older adult drones require 40-101 mg sugar per day. If it is assumed that the honey the drones consume originally came from a nectar source with an average sugar content (*i.e.,* 30%), the nectar requirements of drones is 133-337 mg/day. According to Szolderits and Crailsheim 1993, individual drones consume 0.2275 mg/day when they are 3 days old and older drones (20 days old) consume 0.0002 mg/day. Therefore, the average amount of nectar and pollen consumed by adult drones is 133-337 mg/day. This is similar to the proposed food consumption rate used to represent adult worker bees (*i.e.,* 292 mg/day), suggesting that the adult worker value will also be representative of exposures to adult drones.

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Queens

Queen larvae consume royal jelly that is composed of glandular secretions of nurse bees. According to Dietz and Lambremont 1970, queen larvae consume 40% more food compared to worker larvae during the first 6 days of the larval life stage. Although the consumption rate of queen larvae is higher than worker larvae by a factor of 2.5, the overall exposure to queen larvae is expected to be less than worker larvae because the proposed exposure value is based on the assumption that worker larvae consume pollen and nectar, which has a concentration that is >100 times greater than the expected concentration in food of queen larvae, *i.e.,* royal jelly (Davis and Shuel 1988, Kamel *et al*. (unpublished)) (**Table 1-8**).

As adults, queens are fed a mixture of brood food and honey. Information on the food consumption rates of adult queen bees have not been located. As discussed above, because pesticide concentrations in brood food are expected to be orders of magnitude below concentrations in pollen and nectar, the food consumption rate of adult queens would have to be more than a factor of 100 greater than worker bees in order for the adult worker bee exposure value to be under protective for adult queen bees.

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Appendix 2. Discussion of Potential Pesticide Exposures through Consumption of Contaminated Drinking Water

In order to understand the potential pesticide exposure to bees through consumption of water, it is important to be able to estimate pesticide concentrations in the different types of water bodies from which bees may collect water, as well as the amounts of water bees will collect from each source. Pesticide concentrations are expected to vary among potential sources of drinking water. This appendix includes estimated pesticide concentrations in ponds, puddles and dew, which are all considered relevant sources of water for bees. In estimating the total pesticide exposure a single bee may get from drinking water, there are two major sources of uncertainty: 1) the major source(s) of water used by bees are unknown, and 2) the amount of water a bee will actually drink is unknown. This section estimates potential drinking water intake rates for bees using two different methods that generate results that differ by orders of magnitude, concluding that the method based on water flux rates in a similar species is more reliable. In addition, this section discusses potential exposures through contaminated guttation fluid, which has been examined in the literature in recent years as being a potential drinking water exposure route of concern due to its high (ppm level) concentrations of some pesticides following pesticide treatments. No method has been identified for estimating pesticide concentrations in guttation fluid, so measured concentrations are considered.

Water Uses and Sources

Honey bees collect water for two primary reasons: first, to regulate the temperature within the hive through evaporative cooling to maintain conditions for healthy brood; and second, to use as a part of the preparation of larval brood food by nurse bees (Gary *et al.*, 1978; Winston, 1987; Seeley, 1995; Visscher *et al.* 1996, Kühnholz and Seeley 1997, Nicolson 2009, Kovac *et al.* 2010). It is assumed that when an individual bee's daily water requirement is not met by food (*i.e.,* consumption of honey and nectar), it will drink water to meet that requirement. An average colony will collect approximately 25 L of water annually (Seeley 1995). Unlike pollen and nectar (more precisely, bee bread and honey), water is not stored within the hive (Kovac *et al.* 2010). Therefore, water must be collected by foraging bees when needed by the colony.

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There is no indication that bees prefer a particular water source; however, they are often observed at water sources closest to their hive (Gary *et al.*, 1978). Honey bees have been observed collecting water from a variety of sources, including: streams, ponds, lakes, creeks, marshes and puddles. Bees have also been observed collecting water from grass and plant stalks (Gary *et al.* 1978, Seeley 1995, Kühnholz and Seeley 1997, Schmaranzer, 2000), suggesting that they may collect dew and guttation fluid present on plants. Guttation fluid has been observed during times of honey bee foraging during morning hours (Girolami *et al.* 2009, Lückmann *et al.* 2010) and has been documented as a potential source of water for honey bees (Reetz *et al.* 2011). It is unknown whether honey bees utilize guttation fluid as a primary source of water.

Drinking Water Consumption Rates

This section presents two methods for estimating daily drinking water consumption rates for individual bees. One method estimates the amount of water consumed by forager bees based on observations of bee behavior. The other method uses the water flux rate of the brown paper wasp (*Polistes fuscatus*), a species that is similar to the honey bee, to represent the water flux rate of bee. These two methods yield very different results and have their own strengths and limitations. **Table 2-1** summarizes the two methods, which are also described below.

Table 2-1. Summary of two methods used to estimate drinking water consumption rates of honey bees.

Method	Drinking water consumption (mL/day)	Strengths	Limitations
Estimate from water forager honey bee observations (# trips, amount of water collected and kept)	0.45-1.8	Based on observations for honey bees	Not based on direct measurements, but on observations of honey bee behaviors. Focuses only on one type of worker bee (*i.e.,* water foragers). Parameter values are uncertain. Estimate is 5-20x the amount of water in the body of a bee.
Flux rate of brown paper wasps	0.047	Based on direct measures of the water requirements of wasps, which are assumed to be an appropriate surrogate for the honey bee.	Not based on honey bee data.

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Method based on Honey Bee Observations

The first method for estimating daily water consumption incorporates information describing the amount of water collected and kept by water forager bees in order to estimate their daily needs. In this approach, average amount of water collected per day is calculated by multiplying the average amount of water collected per trip, *i.e.*, 0.030 – 0.060 mL (Visscher *et al.* 1996, Thompson 2010, Seeley 1995) by the number of trips per day, *i.e.*, 50-100 (Seeley, 1995, Johansson and Johansson 1978). The resulting estimate of water collected by water foragers per day is 1.5-6 mL. The estimated amount of water consumed by water foraging bees is calculated by multiplying the amount of water collected by these bees by 30% (this approximate value was reported by Johansson and Johansson 1978). The estimated amount of water consumed by water forager bees is 0.45-1.8 mL/day.

The strength of this range of estimated drinking water rates is that it is based on observations of honey bees foraging for water; however, the estimated range is limited in that it is dependent upon several parameters that are highly variable and uncertain (*e.g.,* Johansson and Johansson 1978 reported an approximate value for how much water a water forager bee will keep). Also, this analysis focuses only on data for water forager bees. It does not consider the water consumption rates of other types of worker bees. In addition, these variables are expected to be impacted by time of year and temperature. For instance, water loads have been documented to increase with increasing ambient temperature (Kovac *et al.* 2010). A final limitation of this approach can be illustrated by comparing the estimated daily water consumption rate to the water content of a bee. The estimate water content of a bee, 0.088 mL, can be estimated by multiplying the body weight, *i.e.,* 0.128 g (Mayer and Johansen 1990) by the water content of an insect, *i.e.,* 69% (USEPA 1993), and the standard density of water, *i.e.,* 1 g = 1 mL. Comparison of the water content of a bee to the water consumption rate estimated using this method indicates that the bees would consume 5 to 21X the amount of water in their bodies. Despite the fact that bees eliminate water during the day, it is unlikely that bees would have to replace the entire amount of water in their bodies 5-20 times a day.

Method based on Brown Paper Wasp Flux Rate

The second method for estimating the amount of water consumed by an individual bee is to use the daily water intake rate (flux rate) of bees, which accounts for water intake from all sources (primarily

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from food and drinking). No flux data have been located for honey bees; however, direct measurements of flux data are available for the brown paper wasp. The brown paper wasp is considered an appropriate surrogate for honey bees for several reasons. First, they are taxonomically related (*i.e.*, both species are in the Hymoneptera order). Second, they are similar in weight to honey bees (worker wasps weigh 0.122 g (Nagy and Peterson 1988), while worker bees weigh 0.128 g (Mayer and Johansen 1990)). Finally, both species are social (colonial) and utilize water for cooling their nests (Nicolson 2009).

According to Nagy and Peterson 1988, brown paper wasp workers require 0.050 mL/day of water and their queens require 0.067 mL/day. The drinking water intake rate can be estimated by subtracting the water intake rate from food from the flux rate. As discussed in **Appendix 1**, adult worker honey bees meet their dietary needs through consumption of nectar, honey, pollen and bee bread. Since the focus of **Appendix 1** was on deriving food consumption rates that could be related to pesticide concentrations in food sources collected by bees (*i.e.,* nectar and pollen), honey and bee bread were assumed to be represented by nectar and pollen. In this case, because the water contents of the different food items differ substantially, it is necessary to distinguish between the food items when estimating the amount of water a bee will consume through food. In this case, it is assumed that pollen and bee bread contribute a negligible amount of water because they have much lower water contents and are consumed at lower rates compared to nectar and pollen. If it is assumed that 1) worker bees consume nectar with 30% sugar content **(Table 1-4 of Appendix 1**) and honey with 82% sugar content (Winston 1987), 2) that the remainder of nectar (*i.e.,* 70%) and honey (*i.e.,* 18%) is represented by water, and that 3) worker bees consume nectar and honey at the rates provided in **Table 2-2**, then the amount of water consumed in the diet differs based on the task of the worker bee and (as expected) whether a bee is consuming honey or nectar. If a worker bee requires 0.050 mL water/day (based on the brown paper wasp flux rate), then food may make up anywhere from 7% to >100% of the worker bee's daily water requirement. Therefore, in order to fulfill their daily water requirements, worker bees must drink a maximum of 0.047 mL per day.

The strength of this estimate is that is based on direct measurements of water requirements of a closely related insect. Although the flux rate incorporated into this method is not based on data for the honey bee, it appears that the brown paper wasp shares enough characteristics with the honey bee to represent an appropriate surrogate. Unlike the first method described in this section, this estimated

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value appears to be reasonable, given that it is less than, but still on the same order of magnitude as the water content of a bee (*i.e.,* 0.088 mL).

Table 2-2. Estimated food consumption rates and resulting amounts of water consumed with food for adult worker bees by task.

Worker Task	Nectar			Honey		
	Food consumption rate (mg/day)*	Water consumed with food (mL/day)	Percent daily water flux consumed with food	Food consumption rate (mg/day)**	Water consumed with food (mL/day)	Percent daily water flux consumed with food
Cell cleaning and capping	60	0.042	84%	22	0.0040	8%
Brood and queen tending (nurse bees)	167	0.1169	234%	61	0.0110	22%
Comb building, cleaning and food handling	60	0.042	84%	22	0.0040	8%
Foraging (pollen)	52	0.0364	73%	19	0.0034	7%
Foraging (nectar)	292+	0.2044	409%	107	0.0192	38%

*Adapted from Rortais *et al.* 2005 using sugar consumption rates and assuming that the sugar content of nectar is 30% (average of data in Table 1-4).
** Adapted from Rortais *et al.* 2005 using sugar consumption rates and assuming that the sugar content of honey is 82% (Winston 1987).
+Analysis described in Appendix 1.

Preferred method for estimating drinking water intake rate

When considering the strengths and limitations of the two methods for estimating a drinking water intake rate for worker bees, the method based on the brown paper wasp flux rate appears to generate a more reliable estimate. Therefore, a drinking water intake rate of 0.047 mL/day will be used in the following section of this appendix to estimate pesticide doses through various sources drinking water.

Estimation of pesticide doses through consumption of different sources of contaminated water

This section explores potential pesticide exposures to honey bees through drinking contaminated water from ponds (adjacent to fields treated with pesticides), puddles (located on treated fields), dew (on crops sprayed with pesticides), and guttation fluid in crops. Concentrations in ponds, puddles and dew are typically estimated using conservative modeling approaches that are based on equilibrium partitioning. In this section, pesticide concentrations are calculated based on an assumed application rate of 1 lb a.i./A (per year). No method has been identified for estimating pesticide concentrations in guttation fluid, so only measured concentrations are considered below. In order to compare the potential pesticide doses received by bees through drinking water to doses received through diet and direct spray, concentrations are translated to a daily dose by multiplying by the daily drinking water intake rate (*i.e.,* 0.047 mL/day). In this approach, for each drinking water source, it is assumed that the bees are consuming 100% of their drinking water from that source. The resulting doses are expressed as μg a.i./bee per 1 lb a.i./A.

Ponds

The GENEEC2 (*GEN*eric *E*stimated *E*nvironmental *C*oncentration) model (version 2) was used to estimate pesticide concentrations in ponds adjacent to treated fields[18]. This model uses the K_{oc} and degradation kinetic data to estimate runoff from a ten-hectare (10-ha) field into a 1-ha by 2-m deep (20,000 m^3) "standard" pond. This model is designed for use as a coarse screen for risk assessments and estimates conservative pesticide concentrations in surface water from a few basic chemical parameters and pesticide label use and application information. In order to derive conservative pesticide concentrations in ponds for a variety of chemicals with different mobilities, it was assumed that the pesticides were

[18] A detailed description of this model is available online at: http://www.epa.gov/oppefed1/models/water/geneec2_description.htm .

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applied via aerial applications and had no degradation. Different K_{oc} values were selected according to the Food and Agriculture Organization (FAO) mobility classification (FAO 2000) in order to estimate concentrations in ponds for chemicals with different mobilities (ranging from highly mobile to immobile; **Table 2-3**). Input parameters for GENEEC2 are provided in **Table 2-4**.

Table 2.3. FAO mobility classifications and corresponding K_{oc} ranges.

FAO mobility class	K_{oc} (L/kg)
Highly mobile	<10
Mobile	10-100
Moderately mobile	100-1,000
Slightly mobile	1,000-10,000
Hardly mobile	10,000-100,000
Immobile	>100,000

Table 2-4. Input parameters used to run GENEEC2 for estimating pesticide concentrations in ponds adjacent to fields.

Parameter Description	Parameter Value	Comments
Application Rate (lb a.i./A)	1	None
Maximum number of applications per year	1	None
K_{oc} (L/kg-oc)	1 10 100 1000 10,000 100,000 1,000,000	Only one K_{oc} value selected for each model run
Soil aerobic metabolic half-life (days)	0	Assume chemical is stable
Is pesticide wetted in?	No	None
Method of application	Aerial	Most conservative
Droplet size distribution	Very fine to fine	Most conservative
Width of no spray zone (feet)	0	Most conservative
Solubility (ppm)	100,000	Set high value to prevent influence of solubility.
Aerobic Aquatic Metabolism half-life (days)	0	Assume chemical is stable
Hydrolysis half-life (days)	0	Assume chemical is stable
Photolysis half-life (days)	0	Assume chemical is stable

Estimated concentrations range from 1.8 to 67 μg a.i./L for chemicals with K_{oc} values ranging 1-1,000,000 L/kg-oc (**Table 2-5**). Assuming that 100% of the bees' drinking water comes from ponds the

corresponding dose range is 0.00008-0031 μg a.i./bee. These values are 3 or more orders of magnitude below the estimated contact or dietary doses proposed for the Tier I screen for foliar spray applications. For soil and seed treatments, the doses through drinking water are 2 orders of magnitude below the dietary doses generated using the recommended Tier I method for estimating exposures resulting from these applications. Based on this information, it appears that pesticide doses received by bees drinking from ponds are insignificant relative to doses received through consumption of food or through direct spray onto the bees.

Table 2-5. Peak estimated concentrations in pond (in μg a.i./L) as estimated using GENEEC2 and resulting doses from 100% of drinking water from ponds.

K_{oc} (L/kg)	Estimated concentration in pond water (µg a.i./L)	Dose (μg a.i./bee)				
		Drinking water	Foliar spray: contact	Foliar spray: diet	Soil application: diet*	Seed treatment: diet
1	67	0.0031	2.7	32	0.62	0.29
10	66	0.0031			0.56	
100	59	0.0028			0.29	
1,000	33	0.0016			0.15	
10,000	11	0.00052			0.084	
100,000	5.2	0.00024			<0.063	
1,000,000	1.8	0.00008			<0.056	

*Calculated assuming $K_{ow} = K_{oc}/0.35$.

Puddles

Pesticide concentrations in puddles located on pesticide-treated fields can be estimated using the Tier I rice model (v. 1.0) (USEPA 2007), with modifications to simulate an on-field puddle rather than a rice paddy. The Tier I rice model relies on an equilibrium partitioning concept to provide conservative estimates of environmental concentrations resulting from application of pesticides to rice paddies. When a pesticide is applied to a rice paddy, the model assumes that it will instantaneously partition between a water phase and a sediment phase, independent of the size of the puddle. The Tier I rice model is represented by **Equation 2-1**. OPP uses this model with parameters that represent a rice paddy (*i.e.,* sediment depth, water depth, bulk density and porosity). This model can be modified to represent a puddle on a treated field using values for these parameters that represent on-field puddles. In this application, it is assumed that puddles may be directly sprayed with a pesticide and that equilibrium is reached instantaneously.

Equation 2-1 $$C_w = \frac{m_{ai}'}{d_w + d_{sed}\left(\theta_{sed} + \rho_b K_d\right)}$$

Where:
C_w = water concentration (µg/L)
m_{ai}' = mass applied per unit area (kg/ha)
K_d = water-soil partitioning coefficient (L/kg) (equivalent to Koc *0.01)
d_{sed}=sediment depth (m)
d_w= water depth (m)
ρ_b= bulk density (kg/m^3)
θ_{sed}=porosity (unitless)

In order to allow for comparisons of pesticide doses through consumption of puddle water to other doses discussed in this paper, values are normalized to 1 lb a.i./A. Therefore, a value of 1.12 kg/ha is used to represent the mass applied per unit area (m_{ai}') for all estimates of the concentration in water (C_w) generated using **Equation 2-1**. A sensitivity analysis was conducted to identify the parameters that would yield high-end estimates of exposure. In this analysis, puddle water depths (d_w) ranged from 0.5 to 6 inches (*i.e.,* 0.013-0.15 m), sediment depth (d_{sed}) was 10-200% of the puddle water depth, bulk density (ρ_b) was set to 1400-1800 kg/m^3 (which is within the range of the standard PRZM scenarios used by EPA to represent fields through the US). Porosity did not influence the estimated concentration in water, therefore it was set to a value of 0.509, which is the default used by the Tier I Rice model. Soil partitioning coefficient (K_d) values were selected to be consistent with the values used to estimate pesticide concentrations in ponds and dew (assuming K_d = 0.01*K_{oc}). The C_w values for different parameter values and are provided in **Table 2-6**. In this table, a K_d of 0.1 L/kg is used to illustrate the difference in sensitivity of model results to the different puddle parameters.

Table 2-6. Sensitivity analysis of puddle model parameters. Highly-mobile chemical (K_d = 0.1 L/kg) used as an example.

d_w (m)	d_{sed} (m)	porosity	pb (kg/m^3)	k_d (L/kg)	C_w (ug/L)	D_{water} (low) (*ug* a.i./bee)	D_{water} (high) (*ug* a.i./bee)
0.013	**0.0013**	**0.509**	**1400**	**0.1**	**5.724166**	**1.4E-04**	**1.0E-02**
0.013	0.013	0.509	1400	0.1	0.608822	1.5E-05	1.1E-03
0.013	0.026	0.509	1400	0.1	0.305491	7.6E-06	5.5E-04
0.013	0.0013	0.509	1800	0.1	4.522298	1.1E-04	8.1E-03
0.013	0.013	0.509	1800	0.1	0.474653	1.2E-05	8.5E-04
0.013	0.026	0.509	1800	0.1	0.237982	5.9E-06	4.3E-04
0.15	0.015	0.509	1400	0.1	0.496094	1.2E-05	8.9E-04
0.15	0.15	0.509	1400	0.1	0.052765	1.3E-06	9.5E-05
0.15	0.3	0.509	1400	0.1	0.026476	6.6E-07	4.8E-05
0.15	0.015	0.509	1800	0.1	0.391932	9.8E-06	7.1E-04
0.15	0.15	0.509	1800	0.1	0.041137	1.0E-06	7.4E-05
0.15	0.3	0.509	1800	0.1	0.020625	5.2E-07	3.7E-05

The high end estimates for all of the selected K_{oc} values (0.01*K_d) are provided in **Table 2-7,** along with conversions of these values to doses received by bees consuming 100% of their daily drinking water from contaminated puddles. Estimated concentrations in puddles are on the same order of magnitude or below that of concentrations estimated in ponds using GENEEC2. Therefore, similar to bees that drink water from ponds, bees that meet 100% of their drinking water needs from puddles (located on treated fields) are expected to receive pesticide doses that are insignificant relative to doses received through consumption of food or through direct spray onto the bees.

Table 2-7. Peak estimated concentrations in puddle (in *µg* a.i./L) as estimated using modified Tier I rice model and resulting doses from 100% of drinking water from puddle.

K_{oc} (L/kg)	Estimated concentration in puddle water (*µg* a.i./L)	Dose (*µg* a.i./bee)				
		Drinking water	Foliar spray: contact	Foliar spray: diet	Soil application: diet*	Seed treatment: diet
1	57	0.0027	2.7	32	0.62	0.29
10	5.7	0.00027			0.56	
100	0.61	0.000029			0.29	
1,000	0.061	0.0000029			0.15	
10,000	0.0061	0.00000029			0.084	
100,000	0.00061	0.000000029			<0.063	
1,000,000	0.000061	0.0000000029			<0.056	

*Calculated assuming Kow = Koc/0.35.

Dew

This analysis relies upon a simple equilibrium partition model based on K_{oc} and the carbon content of plants. The partitioning model, which is depicted in **Equation 2-2**, is a two-compartment model including water and leaf organic carbon into which the pesticide may associate. In past discussions with the SAP, this approach was considered appropriate by the SAP when discussing methods for estimating pesticide exposure to birds (USEPA 2001). The value $C_{dew(t)}$ is the concentration of dissolved pesticide in dew (mg/L). The value $C_{plant(t)}$ is the concentration of pesticide on and in plant leaves (mg/kg (fresh weight)) at time t after application and can be derived from the T-REX model. For a single application of 1 lb a.i./A to foliage, the most conservative $C_{plant(t=0)}$ derived from T-REX is 240 mg a.i./kg-foliage, which corresponds to residues on short grass. The K_{oc} is the organic carbon:water partition coefficient of the pesticide being modeled (in L/kg-oc) and f_{oc} is the fraction of organic carbon in leaves. Donahue *et al.* (1983) have estimated the fraction of organic carbon in dry leaves to be 0.40 for alfalfa, clover, bluegrass, corn stalk, and small grain straw. If it is assumed that the water content of plants is 90% (Raven *et al.* 1999), this is equivalent to 0.04 on a fresh weight basis. Therefore, for this analysis, f_{oc} is set to a value of 0.04.

Equation 2-2 $$C_{dew(t)} = \frac{C_{plant(t)}}{K_{oc} * f_{oc}}$$

Ranges of C_w values in dew generated using different K_{oc} values are provided in **Table 2-8** along with the estimated doses received by forager bees consuming 100% of their daily drinking water from contaminated dew. When considering the proposed Tier I method for estimating exposures of bees to pesticides applied via foliar spray, estimated doses received through consumption of dew are equivalent to diet and contact for chemicals that are mobile to highly mobile (i.e., Koc <100 L/kg, **Table 2-3**). For chemical that are moderately mobile to immobile (*i.e.,* K_{oc} >100 L/kg, **Table 2-3**), the dose received through drinking dew is less substantial compared to doses through the diet or contact. Since this method assumes that the pesticide is applied directly to the foliage and then partitions into dew, this exposure route is not relevant to soil or seed treatments. The results of this analysis indicate that dew may represent a substantial exposure pathway for bees in cases where the following conditions are met: 1) the bees drink 100% of their daily drinking water from contaminated dew, 2) bees consume only 7% of their daily water needs through food, 3) the dew contains a pesticide with a K_{oc} <100 L/kg and 4) the pesticide is applied via foliar spray. Although these results are initially of concern relevant to assessing

exposures of honey bees to pesticides, this exposure route is not incorporated into the Tier I exposure method because the first condition listed above (for describing when dew exposure is substantial) is expected to be unlikely. This is because dew is only expected to be present during a portion of the morning (Kovac *et al.* 2010), and would not be available during the majority of the day. Also, dew is not expected to be present during the hottest parts of the day, which are expected to represent the times when water demand of the individual bee would be at its greatest. In addition, this approach assumes that bees only acquire 7% of their daily water needs from food (condition 2 above). As indicated in **Table 2-2**, bees may consume anywhere from 7% to >100% of their daily water requirement through food. Therefore, for many bees, pesticide doses through consumption of dew may be much less due to lower or non-existent drinking water consumption rates.

Table 2-8. Peak estimated concentrations in dew (in μg a.i./L) as estimated using K_{oc} partitioning model and resulting doses from 100% of drinking water from dew.

K_{oc} (L/kg)	Estimated concentration in dew (μg a.i./L)	Dose (μg a.i./bee)				
		Drinking water	Foliar spray: contact	Foliar spray: diet	Soil application: diet	Seed treatment: diet
1	6000000	280	2.7	32	Not applicable	Not applicable
10	600000	28				
100	60000	2.8				
1,000	6000	0.28				
10,000	600	0.028				
100,000	60	0.0028				
1,000,000	6	0.00028				

Guttation Fluid

Methods are not available to estimate pesticide concentrations in guttation fluid. Although the Briggs' model was considered, comparison of estimated pesticide concentrations in the transpiration stream to empirical measures of pesticides in guttation fluid indicate that this approach is not suitable (because the empirical concentrations are orders of magnitude above the Briggs' estimates). This difference may be attributed to concentration of chemicals in guttation fluid on the outside of the plant due to evaporation of the water content of the fluid and the basis of the Briggs' model on the internal fluid of the plant, which is not expected to concentrate a chemical due to evaporation. Therefore, this section focuses on available empirical data where pesticide concentrations were measured in guttation fluid.

Empirical measurements of pesticide concentrations in guttation fluid are available from 4 studies in the scientific literature as well as 4 registrant-submitted studies. The majority of the data were generated from studies where crops received seed treatments of pesticides; however data are available where pesticide concentrations in guttation fluid were measured from crops where pesticides were applied via foliar spray or soil treatment. Descriptions of these studies are provided in **Appendices 3 and 4**. All but two studies involved seed treatments with neonicotinoid insecticides (specifically clothianidin, imidacloprid and thiamethoxam) and monitoring of pesticides in guttation fluid of young and mature plants. The highest pesticide concentrations reported in these studies ranged from 8100 to 39,000,000 μg a.i./ L (**Table 2-9**). These concentrations are orders of magnitude above the estimated concentrations in ponds, puddles and dew. Individual bees drinking all of their daily drinking water from guttation fluid would receive pesticide doses that are equivalent to or higher than those doses received through direct spray from foliar applications. This would suggest that pesticide exposures through drinking guttation fluid of crops that received treatments of pesticides may be substantial for bees.

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Table 2-9. Pesticide concentrations reported in guttation fluid of crops treated with pesticides and corresponding doses in bees consuming 100% of their daily drinking water from this source.

Chemical	Application method	Crop	Measured concentration (µg a.i./ L)	Dose (µg a.i./bee)	Citation**
Thiamethoxam	Seed treatment	Corn	8,740,000	411	Tapparo *et al.* 2011
Clothianidin	Seed treatment	Corn	6,400,000	301	Lückmann *et* al. 2010
Clothianidin	Seed treatment	Corn	2,240,000	105	Lückmann *et* al. 2010
Imidacloprid	Seed treatment	Corn	2,100,000	99	Tapparo *et al.* 2011
Thiamethoxam	Seed treatment	Corn	1,920,000	90	Tapparo *et al.* 2011
Imidacloprid	Seed treatment	Corn	1,230,000	58	Tapparo *et al.* 2011
Thiamethoxam	Seed treatment	Corn	1,170,000	55	Tapparo *et al.* 2011
Thiamethoxam	Seed treatment	Corn	1,110,000	52	Tapparo *et al.* 2011
Imidacloprid	Seed treatment	Corn	712,000	33	Girolami *et al.* 2009
Clothianidin	Seed treatment	Corn	616,000	29	Tapparo *et al.* 2011
Clothianidin	Seed treatment	Corn	561,000	26	FOAG 2009
Clothianidin	Seed treatment	Corn	379,000	18	FOAG 2009
Clothianidin	Seed treatment	Corn	218,000	10	Tapparo *et al.* 2011
Thiamethoxam	Seed treatment	Corn	184,000	8.6	Tapparo *et al.* 2011
Clothianidin	Seed treatment	Corn	141,000	6.6	Girolami *et al.* 2009
Clothianidin	Seed treatment	Corn	110,000	5.2	Reetz *et al.* 2011
Imidacloprid	Soil treatment	Melon	99,100	4.7	Hoffman and Castle 2012
Thiamethoxam	Seed treatment	Corn	90,200	4.2	Girolami *et al.* 2009
Unnamed chemical #2	Foliar spray	Oilseed rape	38,650*	1.8	Study 2-6
Unnamed chemical #2	Foliar spray	Oilseed rape	8,100*	0.38	Study 2-7

*Normalized to 1 lb a.i./A.
**Summaries of these studies are provided in Appendices 3 and 4.

Although these preliminary conclusions represent a concern for assessing exposures of honey bees to pesticides, there are several factors that limit incorporation of this exposure route into the Tier I screen for all pesticides. First, the conclusion that guttation fluid could represent a substantial exposure route for bees is based on the assumption that bees are collecting 100% of their daily drinking water from this source. As discussed above when considering exposure through consumption of dew, this assumption is unlikely given that guttation fluid is only present during the morning hours of the day (Reetz *et al.* 2011), which are not expected to be the times when the water requirements of an individual bee would be the highest. Second, as discussed in the dew section above, this analysis is based on the assumption that bees only acquire 7% of their daily water needs from food; however, many bees are expected to consume much more water with their food. Therefore, for many bees, pesticide doses through

consumption of guttation fluid may be much less due to lower or non-existent drinking water consumption rates. Third, the importance of guttation fluid to bees as a source of drinking water is unknown. Perhaps guttation fluid will be more important to bees located in areas where surface water is not available (*e.g.,* arid climates), or when it is the closest source of water to the hive in times of need. Finally, it is unclear which pesticides would be present in guttation fluid. Physical chemical properties of a chemical are expected to impact the mobility of a chemical within a plant. The three pesticides that have been reported in guttation fluid after seed or soil treatments all have high solubility and high mobility. Some of the same studies also included seed treatments of fipronil (solubility = 4.2 mg/L; Log K_{ow} = 3.5; K_{oc} = 727 L/kg-oc), which has lower solubility and mobility compared to clothianidin, imidacloprid and thiamethoxam (solubility values range: 327 – 4100 mg/L; Log K_{ow} values range: -0.13 to 1.12; K_{oc} values range: 33-345 L/kg-oc). In these studies, fipronil was not detected in guttation fluid of plants (Girolami *et al.* 2009, Tapparo *et al.* 2011). Therefore, although some but not all pesticides may be present in guttation fluid (*e.g.,* following seed treatments).

Summary of Exposure through Drinking Water

Based on estimated pesticide concentrations in various sources of water potentially consumed by bees and a water flux rate from the brown paper wasp, the results of this analysis indicate that if bees consume the majority of their water from puddles or ponds, the exposures relative to dietary and direct spray are insignificant. If bees drink a substantial amount of water from guttation fluid or dew, conservative exposures may be similar to or even exceed pesticide exposures through the diet or direct spray. Because this indicates a potential concern for assessing exposures of honey bees to pesticides, potential exposures through drinking dew and guttation fluid were investigated further. This investigation concluded that pesticide exposures through dew and guttation fluid are not expected to be as significant when compared to diet because of two primary reasons. First, although the importance of dew and guttation fluid to bees as a source of drinking water is unknown, dew and guttation fluid are only expected to be present during a portion of the morning which would prevent bees from drinking a substantial amount of water from these sources. Second, for many worker bees, pesticide doses through consumption of dew and guttation fluid may be much less due to lower or non-existent drinking water consumption rates (because of higher amounts of water consumed through food). Therefore, pesticide exposure through drinking water is not included in the proposed Tier I exposure route for bees.

Appendix 3. Summaries of empirical studies from unpublished, registrant-submitted studies that were used to evaluate Tier I methods for estimating pesticide exposures

This appendix contains summaries of empirical studies from unpublished, registrant-submitted study reports focusing on pesticide concentrations in pollen, nectar and guttation fluid. The maximum concentrations from these studies were used to evaluate the draft tier I modeling approaches included in the white paper.

Beedle and Harbin 2011

Beedle and Harbin 2011 quantified total imidacloprid residues (imidacloprid + 5-hyrdoxy imidacloprid + imidacloprid olefin) in nectar and leaves collected from cotton plants grown at five locations treated with imidacloprid. The five trials were conducted in clay soils with a drainage classification of "heavy", defined as slow drainage capacity. Plant parts were quantified by HPLC/triple stage quadrupole mass spectrometry (LC/MS/MS) using stable isotope-labeled internal standards. Composite samples were collect of cotton nectar and cotton leaves seven days prior to application of imidacloprid and six days following imidacloprid. Admire Pro® (imidacloprid) was applied to the study sites by drip-line chemigation in 2009 at a rate ranging from 0.18 to 0.38 lbs a.i./A. Each study site received one aerial foliar spray of imidacloprid (Provado® 1.6F) in 2010 during flowering at a rate of 0.063 lbs a.i./A. Residues are summarized in **Table 3-1**.

Table 3-1. Summary of total imidacloprid residues measured in cotton nectar and leaves (ppm)

Media	Application rate (lb a.i./A)	Sampling timing DAT	Total imidacloprid (ppm)	Maximum residue (mg/kg per 1 lb a.i./A)
Nectar	NA	(-7 to -2 DBT)	0.0043	--
Nectar	0.063	6 DAT	0.066	1.1
Leaves	NA	(-7 to -2 DBT)	0.025	--
Leaves	0.063	6 DAT	1.9	30

DAT = days after treatment

Bocksch 2010

In a registrant-submitted study (MRID 47961202), residues of clothianidin and imidacloprid and their metabolites were measured in melon following application as seed treatment. The objective of the study was to determine the levels of clothianidin residues and those of its metabolites thiazolylmethylurea (TZMU) and thiazolylnitroguianidine (TZNG), as well as of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidaclodprid-olefin in melon grown from treated seed. Attention was paid to residues in flower, nectar and pollen. The treatment group received a nominal seed dressing rate of 1.0 mg a.i. clothianidin + 0.33 mg a.i. imidacloprid per seed. Samples of entire young melon plants (above ground plant material) were obtained at the time of transplanting from the greenhouse to the field. Additionally, flowers as well as nectar and pollen from combs were collected during flowering, starting at transplanting and continuing during flowering of the crop. In young treatment plants, residues of c1othianidin, TZMU, TZNG, imidacloprid, imidacloprid-5-hydroxy and imidacloprid-olefin ranged from 0.93 to 11 mg/kg for c1othianidin, from 0.10 to 0.50 mg/kg for TZMU, from 0.05 to 0.2 mg/kg for TZNG, from 0.46 to 2.6 mg/kg for imidacloprid, from 0.03 to 0.16 mg/kg for imidacloprid-5-hydroxy and from 0.02 to 0.06 mg/kg for imidacloprid-olefin. In treated flower samples, the residues of clothianidin ranged from < LOD to 0.0030 mg/kg, the residues of imidacloprid ranged from < LOD to 0.0095 mg/kg. No quantifiable residues of TZMU, TZNG, imidacloprid-olefin, except for a single detection of imidacloprid-5-hydroxy at 0.0010 mg/kg that was reported in the treated flower samples. No quantifiable residues were reported in samples of treated pollen or nectar/honey; however, a phylogenetic analysis was not completed on the pollen sampled from the hives to determine the source of the pollen. Also, it is important to note that residues of clothianidin were detected in control flowers and control young plant ranging from 0.0011 mg/kg to 0.0032 mg/kg, and <LOD to 0.0025, respectively. **Table 3-2** summarizes detected residues in flowers, pollen, nectar, and young plants.

Table 3-2. Summary of Clothianidin, imidacloprid and their metabolites in melon flowers, pollen, nectar and young plant grown from treated melon seed.

Sample Matrix	Range	Clothianidin	TZMU	TZNG	Imidacloprid	Olefin	5-OH	Total Residue CNI's (mg/kg)
Flowers	Min	0.001	<LOD	<LOD	0.0011	<LOD	<LOD	<LOD
	Max	0.003	<LOD	<LOD	0.0095	<LOD	<LOD	0.0126
Pollen	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Max	<LOD	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
Nectar	Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Max	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Plant	Min	0.0011	0.1	0.05	0.0012	0.02	0.03	0.0022
	Max	11	0.5	0.2	2.6	0.06	0.16	14

LOD = level of detection

Bryne *et al.* 2011

Byrne *et al.* 2011 quantified total imidacloprid concentrations (imidacloprid + 5-hyrdoxy imidacloprid + imidacloprid olefin) in nectar that was regurgitated from foraging bees returning to the hive, and nectar that was stored in new comb. This study was conducted in two portions, where the first part confined honey bee colonies within tunnel cage enclosures each containing three flowering citrus trees. The second part of the study situated Honey bee colonies within a large area of treated commercial citrus. Admire Pro® (imidacloprid) was applied at the maximum label rate of 14 fl oz per acre (0.104 fl oz per tree; 0.5 lbs a.i./A) as a trunk drench for both the tunnel and open-field studies. Nectar was sampled from individual flowers using a micro-capillary tube and drawn into the tube by capillary action. For the regurgitated nectar samples, bees were intercepted at the entrance to the hives using a small net and were anesthetize on dry ice, after which individual bees were forced to regurgitate the contents of their crops by pressing gently on the lateral sides of their abdomen region with paddle forceps.

Nectar was also collected from deposits made to new comb within each hive. Hive deposited nectar (uncapped honey) was sampled during the afternoon of day 3 after bees were released within the tunnels. Imidacloprid, imidacloprid olefin and 5-hydroxy imidacloprid were measured in nectar and pollen. Nectar was measured directly by dilution with HPLC mobile phase and the pollen was extracted using QuEChERS methodology. The final extracts were analyzed by LC/MS/MS employing stable isotope internal standards. Samples were not taken overtime, but represent exposure during a 3-day period in April when trees were in bloom. The highest measured concentration of 0.11 ppb was in stored nectar.

Federal Office of Agriculture (FOAG) 2009

In a registrant-submitted study (MRID 47881701) monitoring bee health in Switzerland, residues of clothianidin were measured in guttation fluid collected from corn grown from seeds treated with Poncho® (0.5 mg a.i./seed). In the two trials that were conducted guttation fluid was collected 15 to 50 days and 8-38 days after the seeds were sown. Residues were analyzed using an LC-MS/MS method, and the detection limit in water was 0.10 μg/L. Clothianidin residues in guttation fluid were greatest during the early growth stages of the corn plants, when there was more guttation fluid being released, and decreased as the plants grew. Concentrations of clothianidin ranged from 0.05 to 37 μg a.i./mL.

Freeseman and Harbin 2011

Nine trials were conducted to determine imidacloprid, 5-hydroxy imidacloprid, and imidacloprid olefin residues in pollen and leaves of tomatoes treated by drip chemigation with Admire Pro® in 2009 and in 2010. The sites were planted in soils classified as either heavy (4 sites) or medium (5 sites). Six of nine sites were treated twice in 2010, once at or closely following transplantation, and a second 52 to 57 days later. The application rate was 3.5 fl. oz./acre (0.13 lbs. a.i./acre) per treatment for a total application rate of 7.0 fl. oz./acre (0.25 lbs. a.i./acre). The remaining three sites were treated with 5.0 fl. oz./acre (0.18 lbs. a.i./acre). These applications were made 2 to 25 days following transplanting. All applications were made through drip chemigation via buried lines. Residues were measured in anthers and leaves grown in each soil type. It is important to note that Admire Pro was applied at a use rate of 5.0 to 7.0 fl. oz. acre (0.18 – 0.25 lbs. a.i./acre) or 47% to 66% of the maximum label rate. Results are summarized in **Table 3-3.**

Table 3-3. Summary of Total Imidacloprid in soil (unadjusted) (total imidacloprid includes 5-OH imidacloprid and imidacloprid olefin)

Media	Soil Class (drainage)	Application Rate (lbs ai/A)	Sample Timing (DAT)	Total Imidacloprid Residue (ppm)						
				n	Min	Max	highest average site residue	Median	Mean	STDEV
tomato anthers	Heavy Soil	0.25	72-79	8	0.014	0.03	0.027	0.022	0.021	0.005
tomato anthers	Medium Soil	0.25	79-102	10	0.016	0.054	0.046	0.036	0.034	0.012
Tomato leaves	Heavy Soil	0.25	72-79	8	0.057	0.057	0.12	0.089	0.093	0.026
Tomato leaves	Medium Soil	0.25	79-102	10	0.038	0.038	0.2	0.1	0.11	0.061

Heavy Soil represents soil with slow drainage capacity, medium has moderate drainage capacity

Lückmann *et al.* 2010

In a registrant-submitted study (MRID 479753-01) residues of clothianidin were measured in guttation fluid of corn seedlings grown from seeds treated with Poncho® (0.5 mg a.i./seed) or Poncho Pro® (1.25 mg a.i./seed) under field conditions in Austria. Two regions in Austria were monitored, Baumgartenberg (with 5 sub-regions) and Jennersdorf (with 4 sub-regions), each region containing separate study fields. The seeding rate was *approximately* 2 units/ha (1 unit = 50,000 corn seeds); therefore, the nominal application rates were 50 g a.i./ha (0.0446 lbs a.i./A) for Poncho®, and 125 g a.i./ha (0.112 lbs a.i./A) for Poncho Pro®, respectively. Each study region was monitored regularly for guttation fluid and/or dew, when guttation fluid was observed; up to 3 samples (1 mL volume) were collected. Guttation fluid was observed ≥97% of observation days in the morning in both study regions (May - June 2009); observations of guttation fluid in the evening was much lower (*approximately* 50% of observation days). During the morning hours the presence of guttation fluid and honeybee forager activity coincided, and occurs throughout the period of seedling growth. When an alternative source of water is not accessible to honeybees, they were seen resting on the soil and corn plants close to their hive, perhaps to forage. A small number of honeybees (3-8) were observed collecting guttation fluid from corn plants, more honeybees were observed collecting guttation fluid and/or dew from other plants within the field or along the margin of the field. Residues of clothianidin in guttation fluid collected from both regions typically ranged from 100 - 200 μg a.i./mL, and the highest concentration of clothianidin in guttation fluid was 717 μg a.i./mL.

Maus *et al.* 2004

In this field study, Horse Chestnut trees (*Aesculus hippocastanum*) were injected with Imidacloprid SL 200. Applications were based on the stem diameters and ranged from 1.32-1.76 g ai/tree. No residues of Imidacloprid or the two metabolites were detected above the LOQ in control leaves, control nectar, control fruit or treatment fruit. Imidacloprid and both metabolites were detected in all treatment leaf samples from 2 to 124 days after treatment; peak levels occurred 61 days after treatment and were 2200 μg/kg of imidacloprid, 2313 μg/kg of Hydroxy-metabolite, and 259 μg/kg of Olefin-metabolite (parent maximum was measured in T3, maximum degradate concentrations were measured in a different treatment group, T1). Total imidacloprid (imidacloprid + 5-OH + olefin) in T1 was at a maximum 61 DAT, 4642 μg/kg. Imidacloprid was detected in one replicate control blossom sample (8 μg/kg) 7 days after application; no explanation was provided for this contamination. Residues of

imidacloprid were detected in one treatment blossom sample (7 μg/kg) two days after treatment and for imidacloprid (up to 283 μg/kg) and the Hydroxy-metabolite (up to 7 μg/kg) 7 days after treatment. **Table 3-4** summarizes the total imidacloprid residues in tree leaves in the days following the application.

Table 3-4. Summary of Total imidacloprid residues in Horse Chesnut leaves of trees receiving tree injections.

Replicate	Treatment (g a.i./tree)†	DBH (cm)	ln(Leaf biomass) (kg, dry weight)*	Leaf biomass (dry weight)**	Leaf Biomass (wet weight)***	DAT	Total Imidacloprid (μg/kg)
T1	1.45	23.00	1.59	6.60	32.98	1	ND
T2	1.32	21.00	1.44	5.63	28.15	1	ND
T3	1.51	24.00	1.67	7.10	35.51	1	ND
T4	1.76	28.00	1.94	9.29	46.44	1	ND
T1	1.45	23.00	1.59	6.60	32.98	2	1349
T2	1.32	21.00	1.44	5.63	28.15	2	27
T3	1.51	24.00	1.67	7.10	35.51	2	276
T4	1.76	28.00	1.94	9.29	46.44	2	14
T1	1.45	23.00	1.59	6.60	32.98	33	3102
T2	1.32	21.00	1.44	5.63	28.15	33	1666
T3	1.51	24.00	1.67	7.10	35.51	33	2238
T4	1.76	28.00	1.94	9.29	46.44	33	2882
T1	1.45	23.00	1.59	6.60	32.98	61	4642
T2	1.32	21.00	1.44	5.63	28.15	61	287
T3	1.51	24.00	1.67	7.10	35.51	61	2832
T4	1.76	28.00	1.94	9.29	46.44	61	2156
T1	1.45	23.00	1.59	6.60	32.98	96	1292
T2	1.32	21.00	1.44	5.63	28.15	96	36
T3	1.51	24.00	1.67	7.10	35.51	96	2208
T4	1.76	28.00	1.94	9.29	46.44	96	1396
T1	1.45	23.00	1.59	6.60	32.98	124	615
T2	1.32	21.00	1.44	5.63	28.15	124	454
T3	1.51	24.00	1.67	7.10	35.51	124	625
T4	1.76	28.00	1.94	9.29	46.44	124	1086

† adjusted for trunk diameter (0.06 g a.i./cm stem)
*According to Sollins et al., 1973 (hardwoods)
**Correction for bias when converting from ln to arithmetic units (Sollins *et al.* 1973)
***assuming 80% wet weight is H_2O

Staedtler 2009

In a registrant submitted study (MRID 48298801) residues of clothianidin, and its metabolites thiazolylmethylurea (TZMU) and thiazolylnitroguianidine (TZNG), were measured in pollen harvested from maize plants grown in commercial practice from treated seeds (1.25 mg a.i./seed) in the Upper Rhine Valley in Germany. The objective of the study was to determine the levels of clothianidin residues and those of its metabolites (TZMU and TZNG) in corn pollen collected from plants grown from seeds dressed with clothianidin (Poncho Pro®). Samples were collected approximately 72 days after planting treated seed. A total of 252 samples of pollen were taken from five corn fields planted with clothianidin treated corn seed[19], and an additional 193 samples from bee-derived matricies (bee bread, pollen from pollen traps, and dead bees) were collected during the field sampling phase of the study. Corn pollen sample analysis resulted in 237 samples that contained clothianidin residues >LOQ (94% detection frequency, mean residue of 3.4 μg/kg; 90th %-tile residue of 5.2 μg/kg). The maximum detection in corn pollen was 10.4 μg/kg. Residue analysis of pollen from pollen traps placed at the entrance to bee hives resulted in 28 samples that contained clothianidin residues > LOQ (n=118, 24% detection frequency, mean residue of 1.1 μg/kg, 90th %-tile residue of 2.2 μg/kg). The maximum detection in pollen from pollen traps was 11.4 μg/kg. Dead bee residue analysis resulted in two samples >LOQ (1.2 and 1.1 µg/kg, n=39, mean residue of 0.5 μg/kg, 90th %-tile residue of 1.0 μg/kg). Both of these samples were obtained from hives that were designated to the same field region (Müllheim); it is unclear if the bees were from the same hive. Residue analysis of bee bread resulted in 7 samples >LOQ (n=36, mean residue of 1.0 μg/kg, 90th %-tile residue of 2.0 μg/kg). Residues in pollen are summarized in **Table 3-5**.

Table 3-5. Measured concentrations (μg/kg) of clothianidin in pollen collected in the field and in traps placed at hive entrances.

Site	Mean Clothianidin, μg/kg pollen (ppb) (Field Pollen)	Mean Clothianidin, μg/kg pollen(ppb) (Pollen Traps)
Buhl-Oberbruch	3.906	1.142
Schwanau	2.854	NS
Kippenheim	3.358	1.605
Herbolzheim	3.942	NS
Mullheim	2.942	0.73

[19] Bühl-Oberbruch, Schwanau, Kippenheim, Herbolzheim, Müllheim.

Study 1-1

In a registrant submitted semi-field study, residues of unnamed chemical #1 were measured in matrices relevant to exposure of honeybees. Unnamed chemical #1 (49.1 % w/w) was diluted in water and applied with a foliar spray application to cotton in California at full bloom at three test rates, separately: 0.045 lb a.i./acre (1 or 2 applications at 5-day interval), 0.089 lb a.i./acre (2 applications at 5 day-interval) and 0.134 lb a.i./acre (2 applications at 5-day interval). When spray residues were dry, honeybee colonies were exposed to the treated crop in tunnels (two bee hives per tunnel) for 10 days. Samples of pollen (extracted from flowers), forager honeybees (for subsequent extraction of pollen loads and nectar) and pollen and larvae from the combs were taken and analyzed for unnamed chemical #1 and its major metabolite. Additional measurements on the general conditions and weight of the colonies were recorded before and after exposure to ensure the colonies were in a suitable condition to fulfill the purpose of the experiment. Since bees were not present in the field during application, they were exposed to the treated field following application via contact with foliar residues and oral exposure via consumption, transport and processing of pollen and nectar. **Table 3-6** summarizes the residue data in pollen and nectar measured in cotton. Day zero residue values were used to calculate doses from each food source.

Table 3-6. Unnamed chemical #1 residues (mg ai/kg) in pollen collected from treated cotton plants, pollen collected from foragers, and nectar collected from foragers

Application Rate (lb ai/A)	Application Days		Non-Application Days (D1-D4 & D6-D10)	
	D0	D5	Min	Max
Pollen & Stamen from Plants (mg ai/kg)				
0.045 x 1	1.263	n/a	<0.010	<0.010
0.045 x 2	1.077	2.540	<0.010	0.061
0.089 x 2	6.656	0.691	<0.010	0.057
0.134 x 2	2.612	0.075	0.013	0.070
Pollen from Foragers (mg ai/kg)*				
0.045 x 1	0.127 – 0.187	n/a	0.013	0.222
0.045 x 2	0.173 – 0.226	0.192 – 0.830	0.032	0.296
0.089 x 2	0.512 – 2.782	0.787 – 1.146	0.100	2.262
0.134 x 2	1.209 – 2.218	1.150 – 1.420	0.129	2.226
Nectar from Foragers (mg ai/kg)*				
0.045 x 1	0.022 – 0.033	n/a	<0.010	0.126
0.045 x 2	<0.010	0.045 – 0.049	<0.010	0.043
0.089 x 2	<0.010 – 0.074	0.022 – 0.023	<0.010	0.036
0.134 x 2	0.052 – 0.109	0.022 – 0.044	<0.010	1.006

* range in values from bees collected from hive #1 and #2. n/a = not applicable (no pesticide application)

Study 1-2

In a registrant submitted semi-field study residues of unnamed chemical #1 and its metabolite were investigated in honeybee products (pollen and nectar) and plants following spray applications to *Phacelia tanacetifolia* grown on plots in Niefern-Öschelbronn, Germany. Five treatment groups were investigated in separated tunnels; four treated plots and one control. Unnamed chemical #1 was applied as a foliar spray at rates of 24 g a.i./ha (Treatments 1 and 3; 0.021 lbs a.i./A), and 48 g a.i./ha (Treatments 2 and 4; 0.043 lbs a.i./A). Treatments 1 and 2 were conducted before flowering; treatments 3 and 4 were conducted during flowering and daily bee flight/foraging. The condition of the colonies and the development of the bee brood was assessed before the start of exposure of the honeybees in the tunnels, and determined to be acceptable. For treatments T1 and T2, samples were collected 10, 15, and 16 days after application to the *Phacelia* crop. For treatments T3 and T4, samples were collected at 0, 5 and 6 days after application of the test item. Nectar samples obtained from forager bees (regurgitation samples) and pollen samples were collected via pollen traps at the hive entrance. Results from treatment groups 1 and 2 (T1 and T2; before flowering) showed no residues of unnamed chemical #1. Samples from T3 and T4 showed measureable residues and were conducted during flowering. The results of the analysis of nectar samples from controls, T3 and T4 treatments are provided in **Table 3-7**.

Results of the analyses of pollen samples from pollen traps in these treatments are provided in **Table 3-8.**

Table 3-7. Unnamed Chemical #1 results of nectar analysis at 0 DAT for treatments receiving applications during flowering and foraging.

Timing (DAT)	Treatment	Application rate (g a.i./ha)	Application rate (lb a.i./A)	Residues (mg/kg)
0	C	--	--	ND
0	T3 (during flight)	24	0.021	0.0438
0	T3 (during flight)	24	0.021	0.0462
0	T3 (during flight)	24	0.021	0.0424
0	T4 (during flight)	48	0.043	0.0889
0	T4 (during flight)	48	0.043	0.0548
0	T4 (during flight)	48	0.043	0.0503

DAT = Days after treatment

Table 3-8. Results of analysis of pollen samples from pollen traps 0 DAT

Timing (DAT)	Treatment	Application rate (g a.i./ha)	Application rate (lb a.i./A)	Residues (mg/kg)
0	C	--	--	ND
0	T3	24	0.021	0.29
0	T4	48	0.043	0.809

DAT = Days after treatment

Study 1-3

The nature of the radioactive residue of ^{14}C-unnamed chemical #1 in succeeding crops was studied in a confined crop rotational study. In outdoor test plots in California, ^{14}C-unnamed chemical #1 was applied to bare sandy loam soil at a nominal rate of 600 g a.i./ha. Following aging for plant back intervals (PBIs) of 30, 120, and 365 days, radishes (variety cherry belle), lettuce (variety butter crunch), and wheat (variety summit) were planted. Plots were maintained using standard agricultural practices. Immature radish tops, wheat forage, immature lettuce, and wheat hay (fresh cut and dry cut) were harvested 30, 120, and 365 days after treatment (DAT). Mature radish tops and roots, mature lettuce, wheat straw, and wheat grain were also harvested 30, 120, and 365 DAT.

Samples were extracted and analyzed within ~1.1-4.7 months of harvest. Since they were stored at -20°C and analyzed within 6 months of harvest, supporting freezer storage stability are not required

according to OECD guidelines. Final extraction and analysis of stored samples was conducted to establish stability of samples that were analyzed by GC-MS beyond 6 months of harvest. Selected results from this study for the unnamed chemical #1 and its primary metabolite are shown in **Table 3-9**.

Table 3-9. Sample residue data (mg/kg) for a confined rotational study (860.1850) of CHEMICAL 1 applied to directly to bare soil using a single application of 0.535 lbs a.i./A.

Plant back interval (days)	Mature Radish		Mature Lettuce	Wheat			
	Top	Root	Leaf	Forage	Hay	Straw	Grain
Parent (mg/kg)							
30	0.042	<0.001	0.013	0.010	0.004	0.020	<0.001
120	0.009	<0.001	<0.001	<0.001	0.018	0.106	<0.001
365	0.021	<0.001	0.007	<0.001	<0.001	0.009	<0.001
Primary Metabolite (mg/kg)							
30	0.773	0.135	0.365	0.835	0.658	0.952	0.046
120	0.329	0.077	0.286	0.183	1.076	1.452	0.050
365	0.610	0.047	0.176	0.138	0.455	0.330	0.012

Study 1-4

The registrant submitted field trial data for unnamed chemical #1 in/on barley (hay, grain, straw, and forage) from 27 field trials conducted during 2008, 2009, and 2010 in Australia and New Zealand, Europe, and the USA. Each field trial included one untreated plot and one or two treated plots in which unnamed chemical #1, formulated as a 240 g ai/L suspension concentrate (SC), was applied to barley as two foliar broadcast applications at a target rate of 0.043-0.045 lb a.i./A/application (48-50 g a.i./ha/application), for a total seasonal rate of 0.086-0.090 lb ai/A (96-100 g a.i./ha). Separate plots were established for hay and for grain and straw in the EU and US trials.

To assess residue decline, samples of grain and straw were harvested at 0, 6-7, 13-15, 20-22 and/or 27-28 days (1 Australian, 1 New Zealand, and 7 EU trials, and 1 US trial); residue decline in forage was investigated at one Australian and one New Zealand trial at 0-, 7-, 14-, and 28-day pre-harvest intervals (PHIs).

Barley samples were analyzed for residues of unnamed chemical #1 and its metabolites #1 and #2 using a liquid chromatographic system with tandem mass spectrometers (LC/MS/MS). The method was adequately validated prior to, and in conjunction with, the analysis of the field trial samples, using untreated samples fortified separately with unnamed chemical #1 and metabolites at 0.010-5.0 ppm.

The validated limit of quantitation (LOQ) was 0.010 ppm, and the calculated limit of detection (LOD) was 0.003 ppm (EU and US studies) or 0.005-0.006 ppm (Australia and New Zealand studies) for each analyte in barley grain, straw, forage, and hay.

The maximum storage duration from harvest to extraction for analysis was 281 days. Adequate storage stability data are available to support the storage conditions and durations of barley samples. Results from only trials that included day 0 residue samples are shown in **Table 3-10.**

Table 3-10. Summary of results residue-decline trials for unnamed chemical #1 applied to barley which included day 0 measurements.

Trial (City, State/Province, Country, Year)	Crop/ Variety	Commodity or Matrix	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm fresh weight)[b,c]		
					Chemical 1	Metabolite #1	metabolite #2
Trial B-1 (Feilding, Manawatu, New Zealand, 2009)	Barley / Putney	Forage	0.086 (97)	**0**	1.1	0.02	0.17
				7	0.48, 0.39	0.02, 0.01	0.18, 0.13
				14	0.02	ND	0.02
				28	0.01	ND	(0.006)
		Grain		0	0.12	ND	0.01
				7	0.06	ND	0.01
				14	0.02, 0.03	ND, ND	0.02, 0.02
				28	0.01	ND	0.01
		Straw		**0**	1.3	0.02	0.19
				7	1.0	0.04	0.37
				14	0.03, 0.03	ND, ND	0.03, 0.02
				28	(0.006)	ND	ND
Trial B-2 (Cambooya, Queensland, Australia, 2008)	Barley / Fitzroy	Forage	0.086 (96.6)	**0**	3.3	0.09	0.07
				7	0.22, 0.18	0.03, 0.03	0.02, 0.02
				14	0.16	0.04	0.02
		Grain		0	0.27	0.01	0.01
				7	0.05	ND	0.01
				14	0.05, 0.06	ND, (0.009)	0.01, 0.02
		Straw		**0**	4.3	0.06	0.11
				7	0.24	0.02	0.03
				14	0.18, 0.25	0.03, 0.03	0.03, 0.03

[a] PHI = Pre-harvest Interval; the number of days hay samples were allowed to dry in the field is presented in parentheses.
[b] ND = Nondetectable (residues <LOD). LOQ = 0.010 ppm; LOD = 0.005-0.006 ppm (Australia/New Zealand studies) or 0.003 ppm (EU and US studies). Residues between the LOD and LOQ are presented in parentheses.
[c] Results expressed on a fresh weight basis.

Study 1-5

The registrant-submitted field trial data for unnamed chemical #1 in/on canola/oil seed rape from 21 field trials conducted in Australia, Europe, and the USA and Canada (North American Free Trade area; NAFTA): Each field trial included one treated plot in which unnamed chemical #1, formulated as a 240 g a.i./L suspension concentrate (SC), was applied to canola as two foliar broadcast applications at a target

rate of 0.043-0.045 lb a.i./A/application (~48-50 g a.i./ha/application), for a total seasonal target rate of 0.086-0.090 lb a.i./A (~96-100 g a.i./ha).

To assess residue decline, samples of canola seed were collected from five EU trials (in France, Germany, and Spain), and one US trial (MI) at PHIs of approximately 0, 7, 14, 21, and/or 28 days; additional forage samples were collected from three Australian trials at PHIs of 0, 7, and 14 days.

Samples were analyzed for residues of unnamed chemical #1 and its metabolites metabolite #1 and metabolite #2 using a liquid chromatographic system with tandem mass spectrometers (LC/MS/MS). The method was adequately validated prior to and in conjunction with the analysis of the field trial samples, using untreated samples fortified separately with unnamed chemical #1 and metabolites at 0.01-5.0 ppm for seed, and 0.01-0.65 ppm for forage and stubble. The estimated limit of quantitation (LOQ) was 0.010 ppm and the calculated limit of detection (LOD) was 0.003 ppm (EU and NAFTA studies) or 0.005-0.007 ppm (Australia studies) for each analyte.

The maximum storage duration from harvest to extraction for analysis was 296 days. Adequate storage stability data are available to support the storage conditions and durations of the samples. Results from only trials that included day 0 residue samples are shown in **Table 3-11**.

Table 3-11. Summary of results residue-decline trials for unnamed chemical #1 applied to canola which included day 0 measurements.

Trial (City, State/Province, Country, Year)	Crop/ Variety	Commodity or Matrix	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm fresh weight)[b]		
					Chemical 1	metabolite #1	metabolite #2
Trial C-1 (Wunghnu, Victoria, Australia, 2008)	Canola / Pioneer 46Y20	Forage	0.086 (97.0)	**0**	1.0	0.02	0.03
				7	0.06	0.03	0.02
				13	0.03, 0.07	0.05, 0.07	0.01, 0.04
Trial C-2 (Neville, New South Wales, Australia, 2009)	Canola / Hyola 502RR	Forage	0.086 (96.8)	**0**	1.3	0.04	0.16
				7	0.49	0.04	0.11
				14	0.12, 0.17	0.08, 0.07	0.05, 0.06
Trial C-3 (Hamilton, South Australia, Australia, 2009)	Canola / Hurricane	Forage	0.087 (97.7)	**0**	3.7	0.04	0.08
				7	1.1	0.01	0.07
				14	1.1, 1.5	0.01, 0.02	0.08, 0.11
Trial C-4 (Arradon, Brittany, France, 2010)	Canola / Cokpico	Seed	0.078 (88.1)	0	0.464, 0.683	0.021, 0.026	0.013, 0.016
				7	0.079, 0.061	0.026, 0.017	0.013, (0.007)
				15	0.039, 0.047	0.021, 0.019	0.010, 0.011
				21	0.031, 0.029	0.012, 0.013	ND, ND
				28	0.018, 0.022	(0.008), (0.010)	ND, ND

[a] PHI = Pre-harvest Interval; the number of days hay samples were allowed to dry in the field is presented in parentheses.

[b] ND = Non-detectable (residues <LOD). LOQ = 0.010 ppm; LOD = 0.005-0.007 ppm (Australia studies) or 0.003 ppm (EU and US studies). Residues between the LOD and LOQ are presented in parentheses.

Study 1-6

The registrant-submitted field trial data for unnamed chemical #1 in/on wheat (forage, hay, grain, and straw) from 38 trials conducted during the 2008-2009 season in Australia, New Zealand, Brazil, Europe, the USA and Canada. Each field trial included one or three treated plots in which unnamed chemical #1, formulated as a 240 g a.i./L suspension concentrate (SC), was applied to wheat as two foliar broadcast applications at a target rate of 0.043-0.045 lb a.i./A/application (48-50 g a.i./ha/application), for a total seasonal rate of 0.086-0.090 lb ai/A (96-100 g a.i./ha). Separate plots were established for forage, hay, and for grain and straw in the Brazil, EU, and US/Canadian trials. In addition, three separate plots were established in two EU trials (Germany and France) to examine residue decline in grain and straw; at these plots, applications were made earlier in the season to plants at earlier growth stages. At three Australian sites, two Brazilian sites, eight EU sites (France, Germany, Italy, Poland, Spain, and the UK), and two US/Canadian sites (NE and ON), samples were collected at PHIs of 0, 3-4, 6-10, 13-16, 20-22, and/or 27-29 days to assess residue decline.

Wheat samples were analyzed for residues of unnamed chemical #1 and its metabolites metabolite #1 and metabolite #2 using a liquid chromatographic system with tandem mass spectrometers (LC/MS/MS). The method was adequately validated prior to and in conjunction with the analysis of the field trial samples, using untreated samples fortified separately with unnamed chemical #1 and metabolites at 0.01-5.00 ppm. The calculated limit of quantitation (LOQ) was 0.010 ppm, and the calculated limit of detection (LOD) was 0.003 ppm (EU, Brazil, and US and Canada studies) or 0.005-0.006 ppm (Australia and New Zealand studies) for each analyte in wheat forage, hay, grain, and straw.

The maximum storage duration from harvest to extraction for analysis was 354 days, with the exception of two forage samples (one treated, one untreated) which were analyzed 737 days after harvest. Adequate storage stability data are available to support the storage conditions and durations of wheat samples. Results from only trials that included day 0 residue samples are shown in **Table 3-12**.

Table 3-12. Results from Study 1-6.

Trial ID (City, State/ Province, Country, Year)	Crop/ Variety	Commodity or Matrix	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm fresh weight)[b,c]		
					Chemical 1	metabolite #1	metabolite #2
Trial W-1 (Maules Creek, Australia, 2008)	Wheat/ Sunstate	Forage	0.086 (97)	**0**	1.6	0.02	0.05
				8	1.4[f], 0.50[f]	0.03[f], (0.009)[f]	0.16[f], 0.08[f]
				16	0.16	(0.008)	0.02
				28	0.10	(0.006)	0.02
		Grain		0	0.16	ND	(0.006)
				8	0.03	ND	(0.009)
				16	0.02, 0.01	ND, ND	0.01, (0.008)
				28	0.01	ND	0.01
		Straw		**0**	2.4[f]	0.03[f]	0.07[f]
				8	1.7	0.02	0.21
				16	0.20, 0.37	(0.006), 0.01	0.03, 0.03
				28	0.09	ND	0.02
Trial W-2 (York, Australia, 2008)	Wheat/ Yitpi	Grain	0.084 (94.3)	0	0.04	ND	ND
				7	0.05	ND	(0.005)
				14	0.04, 0.03	ND, ND	(0.007), (0.006)
				28	0.03	ND	0.01
		Straw		**0**	1.8	0.04	0.09
				7	1.8	0.06	0.11
				14	1.4, 1.4	0.04, 0.04	0.12, 0.11
				28	1.2	0.04	0.10

Trial ID (City, State/ Province, Country, Year)	Crop/ Variety	Commodity or Matrix	Total Rate lb a.i./A (g a.i./ha)	PHI[a] (day)	Residues (ppm fresh weight)[b,c]		
					Chemical 1	metabolite #1	metabolite #2
Trial W-3 (Palmerston North, Manawatu, New Zealand, 2009)	Wheat/ Sage	Grain	0.086 (97)	0	0.24	(0.005)	(0.007)
				7	0.01	ND	(0.007)
				14	0.01, (0.007)	ND, ND	(0.007), ND
				28	(0.008)	ND	0.01
		Straw		**0**	1.8	0.06	0.05
				7	0.11	(0.006)	0.02
				14	0.06, 0.05	ND, ND	0.03, 0.02
				28	0.06	ND	(0.006)
Trial W-4 (Mogi Mirim, SP, Brazil, 2008)	Wheat/ IAC 375	Forage	0.085 (95.7)	**0**	0.5818	0.0342	0.0588
				3	0.2899	0.0440	0.0964
				7	0.0949	0.0365	0.1321
				14	0.0106	0.0326	0.1092
				21	ND	0.0255	0.0787
		Hay	0.091 (102)	**0 (2)**	2.0328	0.0694	0.1596
				3 (1)	0.6782	0.0745	0.2221
				7 (2)	0.2604, 0.2651	0.0859, 0.0830	0.2771, 0.2661
				14 (2)	0.0472	0.1055	0.2562
				21 (2)	0.0202	0.0821	0.2430
Trial W-5 (Rolandia, PR, Brazil, 2008)	Wheat/ Coodetec 104	Forage	0.093 (105)	**0**	2.5874	0.0328	0.1519
				3	0.8017	0.0393	0.2702
				7	0.3829, 0.3945	0.0240, 0.0344	0.3594, 0.3414
				14	0.0218	(0.0065)	0.3138
				21	0.0104	0.0154	0.1792
		Hay	0.093 (105)	**0 (2)**	3.1285	0.0726	0.1671
				3 (2)	0.9807	0.0491	0.2645
				7 (2)	0.5048, 0.5600	0.0358, 0.0393	0.2625, 0.2719
				14 (2)	0.1962	0.0211	0.1499
				21 (2)	0.0513	(0.0087)	0.0466

[a] PHI = Pre-harvest Interval; the number of days hay samples were allowed to dry in the field is presented in parentheses.

[b] ND = Non-detectable (residues <LOD). LOQ = 0.010 ppm; LOD = 0.005-0.006 ppm (Australia/New Zealand studies) or 0.003 ppm (EU, Brazil and US/Canada studies). Residues between the LOD and LOQ are presented in parentheses.

[c] Samples were harvested late due to late-season rainfall which slowed ripening/dry-down of the crop.

Study 2-1

In a registrant-submitted semi-field study, residues of unnamed chemical #2 were investigated in nectar and pollen from honey bees in *Phacelia tanacetifolia* (referred to as "*Phacelia*") fields treated with foliar applications of unnamed chemical #2. The study consisted of three treatment (tunnels) groups (two treatments and one control). The tunnel tents (5.0 m × 20.0 m and a height of 3.5 m) were placed over

plots of *Phacelia* and were covered with light plastic gauze. Honey bee colonies were established in the enclosures prior to the second applications of T1 and T2. A *Phacelia* crop area of 90 m^2 was available for foraging in each tunnel. T1 was applied at a rate of 10 g a.i./ha (0.0089 lb a.i./A) and T2 was applied at a rate of 100 g a.i./ha (0.089 lb a.i./A). Two applications were applied to the crop in each treatment group; the first was approximately two weeks before flowering. The second applications in T1 and T2 and the water control applications were performed during full flowering and during daily bee flight (*i.e.,* when foraging activity was ≥5 bees/m^2). One healthy, disease-free and queen-right bee hive with 10 combs was used for each treatment group and for the controls. The colonies were set up in the tunnels four days before the second applications in T1 and T2. **Tables 3-13 and 3-14** summarize the maximum residues measured in pollen and nectar 1 day after the second application.

Table 3-13. Summary of unnamed chemical #2 residues measured in pollen collected from forager bees sampled 1 day after the 2nd application.

Treatment	Application rate in g a.i./ha (lb a.i./A)	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
C	0	ND	--
T1	10 (0.0089)*	338.0	38
T2	100 (0.089)*	1718	19

[a]DAA = days after 2nd application
ND = not detected
* Applications made during full flowering and during daily bee flight.

Table 3-14. Summary of unnamed chemical #2 measured in nectar collected from forager bees (collected 1 day after the second application).

Treatment	Application rate in g a.i./ha (lb a.i./A)	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
C	0	ND	ND
T1	10 (0.0089)*	NA	NA
T2	100 (0.089)*	54.25	0.61

NA = not analyzed – sample collection vessel failure.
ND = not detected
* Applications made during full flowering and during daily bee flight.

Study 2-2

In a registrant-submitted field study, residues of unnamed chemical #2 were measured in pollen (anthers) and nectar in melon flowers treated by foliar spray to the flowering melon crop at a rate of 90 g a.i./ha (0.08 lb a.i./A). Two treatment groups were conducted with a separate control, both received the same application rate and same interval between each application (7 days), however T1 received applications during an early growth stage prior to flowering, and T2 during early flowering. The tables below summarize the results of residues measured in pollen and nectar samples collected 1 day after

the second application. The nectar was extracted from the flowers with the capillary method using micropipettes (10 µL). The pollen was sampled by cutting the anthers from blossoms with scissors. In nectar samples from T1, there were no detected residues of unnamed chemical #2 or of any of the metabolites. There were no detected residues in any of the nectar control samples. In the anther samples (for pollen) from T1, there were no residues above LOQ of unnamed chemical #2 or any metabolite in the test item treated samples or the control samples. In T2, unnamed chemical #2 was detected in nectar samples at 6.084 and 13.36 µg ai/kg (median = 9.722 µg ai/kg), and at 20.25 and 97.01 µg ai/kg (median = 58.63 µg ai/kg) in pollen (anther) samples. There were also no detections of parent or metabolites in the control. **Tables 3-15** and **3-16** summarize the results of the analysis of nectar and pollen samples taken 1 day after the 2nd application.

Table 3-15. Residues of unnamed chemical #2 in nectar from melon flowers (µg/kg) 1 day after 2nd application

Treatment	Application rate in g a.i./ha (lb a.i./A)	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
C	--	ND	--
T1	90 (0.08)	ND	--
T2	90 (0.08)	13.36	0.167

Table 3-16. Residues of unnamed chemical #2 in pollen (anthers) from melon flowers (µg/kg) 1 day after 2nd application

Treatment	Application rate in g a.i./ha (lb a.i./A)	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
C	--	ND	--
T1	90 (0.08)	ND	--
T2	90 (0.08)	97.01	1.21

Study 2-3

In a registrant-submitted field study, the magnitude of potential residues of unnamed chemical #2 in pollen was assessed in tomato by residue analysis of anthers from tomato flowers. The study was conducted in Quinto, Province Zaragoza in Spain in May and June 2010. The study included three treatment groups: two treatment groups (T1 and T2) with two applications of the test item 48 and 40 days before first sampling in T1, and 6 and 1 days before first sampling in T2, respectively. The applications were carried out at a rate of 90 g ai/ha. (0.08 lb a.i./A). The third group was the control. The applications were performed 48 days and 40 days before first sampling in T1 (representing treatment at

early growth stage) and 6 days and 1 day before first sampling of tomato anthers in T2 (representing treatment just before flowering). Residue analysis was carried out on samples of anthers from the treated tomato flowers. Samples of anthers were taken on two sampling days after the second application in T1 and T2. The first samples were taken at the beginning of flowering; the second samples were taken two days later. The anthers (for pollen) were sampled by cutting entire anthers from the flowers with scissors. Early growth stage sampling (T1) showed no residues of unnamed chemical #2 or its metabolites. In T2 (early flowering), unnamed chemical #2 was detected at 15.58 and 103.1 µg/kg (median = 59.34 µg/kg). No parent or metabolite residues were detected in the control samples. **Table 3-17** summarizes the results of samples taken after the 2nd application in both treatment groups and the control group.

Table 3-17. Residues of unnamed chemical #2 in pollen (anthers) from tomato flowers (µg/kg) 1 day after 2nd application

Treatment	Application rate in g a.i./ha (lb a.i./A)	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
C	--	ND	--
T1	90 (0.08)	ND	--
T2	90 (0.08)	103.1	1.29

Study 2-4

In a registrant submitted semi-field study, residues of unnamed chemical #2 were measured in nectar and pollen collected from honey bees in melon fields treated with three drip irrigation applications of unnamed chemical #2 at a rate of 100 g a.i./ha (0.089 lb a.i./A). The study consisted of one treatment (with three replicates) and one control (no replicates). The first drip application was performed on flowering melon, followed by two more applications with 7 day application intervals between the three applications. The first application was made at 8 am, which may coincide with bee flight. The second application was made between 10:30 and 11 am, which corresponded with bee flight. The third application was made between 9:30 and 10 pm, which does not correspond to bee flight. Honeybee colonies were placed inside tunnels containing treated or untreated melon at early flowering, 5 days before the third application. **Table 3-18 and 3-19** summarize the results of the residue analysis of nectar and pollen sampled from foraging bees.

Table 3-18. Summary of unnamed chemical #2 residues measured in pollen from forager bees (g/kg) sampled after the 2nd application.

Treatment	Application rate g a.i./ha (lb a.i./A)	DAA[a]	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
Control	--	+4	ND	--
Replicate 1	100 (0.089)	+4	5.126	0.06
Replicate 2	100 (0.089)	+4	7.999	0.09
Replicate 3	100 (0.089)	+4	12.06	0.14
Control	--		ND	--
Replicate 1	100 (0.089)	+7	8.455	0.10
Replicate 2	100 (0.089)	+7	11.17	0.13
Replicate 3	100 (0.089)	+7	5.631	0.06

[a]DAA = days after third application

ND = Not detected (<LOD, level of detection, <1.25 µg/kg)

Table 3-19. Summary of unnamed chemical #2 measured in nectar from forager bee stomach contents (honey stomach contents).

Treatment	Application rate g a.s./ha (lb a.i./A)	DAA[a]	Concentration of unnamed chemical 2 (µg a.i./kg)	Concentration of unnamed chemical 2 (mg/kg per 1 lb a.i./A)
Control	--	+3	ND	--
Replicate 1	100 (0.089)	+3	<LOQ	--
Replicate 2	100 (0.089)	+3	<LOQ	--
Replicate 3	100 (0.089)	+3	ND	--
Control	--	+7	ND	--
Replicate 1	100 (0.089)	+7	26.23	0.26
Replicate 2	100 (0.089)	+7	<LOQ	--
Replicate 3	100 (0.089)	+7	13.16	0.15

LOQ = Level of quantification, 5.0 µg/kg

ND = Not detected (<LOD, level of detection, <1.25 µg/kg)

Study 2-5

In a registrant-submitted field study, residues of unnamed chemical #2 were investigated in nectar and pollen of canola following seed treatments with unnamed chemical #2. Pollen and nectar were collected from the plots during the flowering stage for the canola plants. Each treated plot was planted to treated canola seed at a rate of approximately 7.9 kg/ha (7.0 lb/acre), equivalent to 0.07 lb ai/A (78.6 g ai/ha/season). Four treatment trials were conducted in Canada. No residues of unnamed chemical #2 or its metabolites were detected in pollen or nectar sampled during flower of canola grown from seeds treated with unnamed chemical #2.

Study 2-6

In a registrant-submitted study, concentrations of unnamed chemical #2 in guttation fluid were studied under field conditions following two applications of unnamed chemical #2 applied at 90 g a.i./ha (0.08 lb a.i./A). The study was conducted near Tübingen in Southern Germany. The study included three treatment groups, T1 and T2 receiving applications during different times of bee flight, and the control which received no treatment. The first spray application in the test item treatments T1 and T2 was performed onto fields of nonflowering *B. napus* L. The second application in these groups was performed on flowering *B. napus* L. In the treatment T1 the second application was carried out in the evening, after daily honey bee-flight. In the treatment T2 the second application was carried out on the following day during daily honey bee-flight. Samples of guttation fluid were taken from surface of leaves of treated plants. The results from the analysis of the guttation fluid are summarized below. In the treatment T1, unnamed chemical #2 was found in guttation fluid at levels of 41.09 to 2117 µg ai/kg (median = 1079 µg ai/kg) after the first spray application and at levels of 212.7 to 3092 µg ai/kg (median = 1652 µg ai/kg) after the 2nd spray application. The metabolites were observed at concentrations ranging 7.2 - 45.82 µg/kg. In the treatment T2, unnamed chemical #2 was found in guttation fluid at levels of 16.13 to 1355 µg ai/kg (median = 1277 µg ai/kg) after the first spray application and at levels of 278.6 to 1641 µg ai/kg (median = 959.8 µg ai/kg) after the 2nd spray application (**Table 3-20**). The metabolites were observed at levels that ranged from one order of magnitude to greater than two orders of magnitude below the parent. There were no detectable residues of unnamed chemical #2 or any of the metabolites in guttation fluid from the control at any of the sampling days.

Table 3-20. Summary of residues of unnamed chemical #2 in guttation fluid.

Treatment	Application rate g a.i./ha (lb a.i./A)	DAA[a]	Concentration of Unnamed chemical #2 (µg/kg)	Concentration of unnamed chemical #2 (µg/L per 1 lb a.i./A)[**]
Sampling after 1st application				
C	--	+2	ND[b]	--
C	--	+3	ND	--
C	--	+20	ND	--
T1	90 (0.08)	+2	2117	26,000
T1	90 (0.08)	+3	NS	--
T1	90 (0.08)	+20	41.09	510
T2	90 (0.08)	+2	1355	17,000
T2	90 (0.08)	+3	1277	16,000
T2	90 (0.08)	+20	16.13	200
Sampling after 2nd application				
C	--	+2	ND	--
C	--	+3	ND	--
T1	90 (0.08)	+2	3092	39,000
T1	90 (0.08)	+3	212.7	2,700
T2	90 (0.08)	+2	1641	21,000
T2	90 (0.08)	+3	278.6	3,500

[a]DAA = days after application
ND = <1.25 µg/kg
NS = Not sampled
[**]Assumes that guttation fluid density is equivalent to that of water (i.e., 1 kg = 1 L)

Study 2-7

In a registrant-submitted study, concentrations of unnamed chemical #2 in guttation fluid were studied under field conditions following two applications of unnamed chemical #2 applied as a foliar spray at 90 g a.i./ha (0.08 lb a.i./A). The study was conducted Benfeld in Northern France. The study included three treatment groups, T1 and T2 receiving applications during different times of bee flight, and the control which received no treatment. The first spray application in the test item treatments T1 and T2 was performed onto fields of non-flowering *B. napus* L. The second application in these groups was performed on flowering *B. napus* L. In the treatment T1, the second application was carried out in the evening, after daily honey bee-flight. In the treatment T2, the second application was carried out on the following day during honey bee-flight. Samples of guttation fluid were taken from surfaces of leaves of treated plants. These results are described below. In treatment 1 (T1) residues of unnamed chemical 2 in guttation fluid were at 349.5 µg ai/kg five days after the first spray of the test item and at 534.8 and 194.3 µg ai/kg (median = 364.55 µg ai/kg) at the samplings on DAA+2 and DAA+6, after the second spray of the test item. Quantifiable residues of two of six metabolites were found only at the sampling on DAA+2 at low levels, slightly above the LOQ (approximately 5 µg/kg). In treatment 2 (T2) residues of

unnamed chemical 2 in guttation fluid were at 585.2 μg ai/kg at the first sampling and at 647.1 and 108.8 μg ai/kg (median = 377.95 μg ai/kg) on DAA+2 and DAA+6, respectively (**Table 3-21**). Residues of three of six metabolites were found after the first sampling at low levels, slightly above the LOQ. Residues of two of six metabolites were found on DAA+2 at low levels, slightly above the LOQ.

Table 3-21. Summary of residues of unnamed chemical #2 in guttation fluid.

Treatment	Application rate g a.i./ha (lb a.i./A)	DAA[a]	Concentration of Unnamed chemical #2 (μg/kg)	Maximum residue (µg/L per 1 lb a.i./A)[**]
Sampling after 1st application				
C	--	+5	ND	--
T1	90 (0.08)	+5	349.5	4,400
T2	90 (0.08)	+5	585.2	7,300
Sampling after 2nd application				
C	--	+1	ND	--
T1	90 (0.08)	+2	534.8	6,700
T1	90 (0.08)	+6	194.3	2,400
T2	90 (0.08)	+2	647.1	8,100
T2	90 (0.08)	+6	108.8	1,400

[a]DAA = days after application
ND = <1.25 µg/kg
NS = Not sampled
[**]Assumes that guttation fluid density is equivalent to that of water (i.e., 1 kg = 1 L)

Appendix 4. Summaries of empirical studies from the scientific literature that were used to evaluate Tier I methods for estimating pesticide exposures

This appendix contains summaries of empirical studies from the scientific literature reporting pesticide concentrations in pollen, nectar, on bees and in guttation fluid. The maximum concentrations from these studies were used to evaluate the draft tier I modeling approaches included in the white. These studies are listed in **Table 4-1.**

Table 4-1. Studies from the scientific literature that are summarized in this appendix. Data from these studies are used to evaluate Tier I exposure assessment methods described in the white paper.

Citation	Samples collected	Chemical(s)	Application Type(s)	Crop(s)
Choudhary and Sharma (2008)	Nectar, pollen	Endosulfan, *lambda* Cyhalothrin and Spiromesifen	Foliar spray	Mustard
Choudhary and Sharma (2008)	Nectar, pollen	Imidacloprid	Seed treatment	Mustard
Delabie et al. (1985)	Bees, flowers, leaves	Cypermethrin	Foliar spray	Oilseed rape
Dively and Kamel (2012)	Nectar, pollen, leaves	Imidacloprid, Dinotefuran, Thiamethoxam or Oxamyl	Soil application, foliar spray, seed treatment	Pumpkin
Fries and Wibran (1987)	Pollen	Cypermethrin and unidentified pyrethroid	Foliar spray	Oilseed rape
Girolami *et al.* (2009)	Guttation fluid	Imidacloprid, Clothianidin, Thiamethoxam, and Fipronil	Seed treatment	Corn
Hanny and Harvey (1982)	Pollen, bees	Carbaryl	Foliar spray	Corn
Hoffman and Castle (2012)	Guttation fluid	Imidacloprid	Soil application	Cantaloupes
Koch and Weisser (1997)	Bees	Sodium-fluorescein (tracer)	Foliar spray	Apple and *Phacelia tanacetifolia*
Lord *et al.* (1968)	Nectar	Dimethoate	Soil drench	*Fuchsia sp.,* Nasturtium (*Tropaeolum sp.)*
Reetz *et al.* (2011)	Guttation fluid	Clothianidin	Seed treatment	Corn
Škerl et al. (2009)	Pollen	Diazinon, Thiacloprid	Foliar spray	Apple
Stoner and Eitzer 2012	Pollen, nectar, leaves	Imidacloprid, Thiamethoxam	Soil application	Squash
Tapparo *et al.* (2011)	Guttation fluid	Thiamethoxam, Clothianidin, Imidacloprid, and Fipronil	Seed treatment	Corn
Wallner (2009)	Pollen, nectar	Boscalid, Prothioconazol	Foliar spray	Oilseed rape
Wallner (2009)	Pollen, nectar	Clothianidin	seed treatment	Oilseed rape

Choudhary and Sharma 2008

In a field study involving applications of several pesticides to flowering mustard (*Brassica juncea*), Choudhary and Sharma 2008 quantified concentrations of those pesticides in nectar and pollen. Endosulfan, *lambda* cyhalothrin and spiromesifen were applied via foliar spray at rates of 0.468, 0.067 and 0.201 lb a.i./A, respectively. In addition, imidacloprid was applied as a seed treatment at a rate of 0.019 lb a.i./A (**Table 4-2**). Foliar spray applications were made during two separate years (2003-2004 and 2004-2005) when 50% of mustard plants were flowering. Nectar was collected from bees that were observed foraging on the treated fields (3 replicate fields). Samples were collected at the time of the application and several time periods (1, 4, 8, 24, 48, 72, 120 and 240 hours after application). Concentrations of endosulfan, *lambda* cyhalothrin and spiromesifen were quantified using gas chromatographic analysis; while HPLC was used to quantify imidacloprid. Measured residues of endosulfan, *lambda* cyhalothrin and spiromesifen were highest at the time of application (**Tables 4-3**, **4-4** and **4-5**). Imidacloprid was not detected in pollen or nectar at any time point included in the study (Level of Detection = 10 ppb).

Table 4-2. Application rates and methods of four chemicals included in this study.

Chemical	Application method	Application rate (lb a.i./A)	Application rate (kg a.i./ha)
Endosulfan	Foliar spray	0.468	0.525
Lambda cyhalothrin	Foliar spray	0.067	0.075
Spiromesifen	Foliar spray	0.201	0.225
Imidacloprid	Seed treatment	0.019	0.021

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Table 4-3. Total endosulfan (includes endosulfan I and II) residues measured in nectar during two different field trials. Values represent average of 3 samples measured using Gas Chromatograph Method.

Time (hour after application)	Reported Concentration (mg a.i./kg)		Normalized concentration (mg a.i./kg per 1 lb a.i./A)	
	Nectar	Pollen	Nectar	Pollen
2003-2004				
0	1.825	2.224	3.90	4.75
1	1.551	1.553	3.31	3.32
4	1.333	1.235	2.85	2.64
8	1.098	0.892	2.35	1.91
24	0.919	0.745	1.96	1.59
48	0.715	0.398	1.53	0.850
72	0.313	0.125	0.669	0.267
120	0.010	ND	0.021	ND
240	ND	ND	ND	ND
2004-2005				
0	1.614	2.127	3.45	4.54
1	1.493	1.412	3.19	3.02
4	1.214	1.102	2.59	2.35
8	0.998	0.792	2.13	1.69
24	0.865	0.592	1.85	1.26
48	0.600	0.308	1.28	0.658
72	0.286	0.097	0.611	0.207
120	0.011	ND	0.024	ND
240	ND	ND	ND	ND

ND = not detected

Table 4-4. *Lambda* cyhalothrin residues measured in nectar during two different field trials. Values represent average of 3 samples measured using Gas Chromatograph Method.

Time (hour after application)	Reported Concentration (mg a.i./kg)		Normalized concentration (mg a.i./kg per 1 lb a.i./A)	
	Nectar	Pollen	Nectar	Pollen
2003-2004				
0	0.909	1.672	13.6	25.0
1	0.691	1.264	10.3	18.9
4	0.567	1.023	8.46	15.3
8	0.453	0.806	6.76	12.0
24	0.271	0.437	4.04	6.52
48	0.163	0.197	2.43	2.94
72	0.012	0.043	0.18	0.64
120	ND	ND	ND	ND
240	ND	ND	ND	ND
2004-2005				
0	0.836	1.612	12.5	24.1
1	0.660	1.161	9.85	17.3
4	0.540	0.951	8.06	14.2
8	0.450	0.771	6.72	11.5
24	0.253	0.442	3.78	6.60
48	0.134	0.163	2.00	2.43
72	0.004	0.011	0.06	0.16
120	ND	ND	ND	ND
240	ND	ND	ND	ND

ND = not detected

Table 4-5. Spiromesifen residues measured in nectar during two different field trials. Values represent average of 3 samples measured using Gas Chromatograph Method.

Time (hour after application)	Reported Concentration (mg a.i./kg)		Normalized concentration (mg a.i./kg per 1 lb a.i./A)	
	Nectar	Pollen	Nectar	Pollen
2003-2004				
0	1.452	2.101	7.22	10.5
1	1.121	1.437	5.58	7.15
4	0.801	1.151	3.99	5.73
8	0.705	0.950	3.51	4.73
24	0.508	0.719	2.53	3.58
48	0.257	0.317	1.28	1.58
72	0.094	0.002	0.47	0.01
120	ND	ND	ND	ND
240	ND	ND	ND	ND
2004-2005				
0	1.413	1.827	7.03	9.09
1	1.011	1.242	5.03	6.18
4	0.784	1.037	3.90	5.16
8	0.594	0.900	2.96	4.48
24	0.491	0.592	2.44	2.95
48	0.224	0.296	1.11	1.47
72	0.083	0.004	0.41	0.02
120	ND	ND	ND	ND
240	ND	ND	ND	ND

ND = not detected

Delable *et al.* 1985

In this field study, 8 hives, divided into 2 apiaries, were located adjacent to an oilseed rape (*Brassica sp.*) field treated with 0.045 lb a.i./A (0.050 kg a.i./ha) cypermethrin. Dead bees were collected using Todd traps placed at the hive entrances. Bees from two hives were analyzed for cypermethrin residues on the day of the application (day 0) and for the following 3 days. The reported data are provided in **Table 4-6**. The highest reported concentration in bees was 0.95 mg a.i./kg, which translates to a dose of 2.7 µg a.i./bee per 1 lb a.i./A. The results of this study are limited by a small number of replicates (*i.e.*, only two apiaries) and variability among individual samples (it is presumed that the reported values represent means of samples collected from hives); however, these data are useful in characterizing the concentration of cypermethrin on bees that were potentially directly sprayed and foraged in the treated area.

Table 4-6. Concentrations of cypermethrin measured on dead bees after foraging on treated field.

Day	Apiary #	Highest reported Concentration* (mg a.i./kg)	Normalized concentration (mg a.i./kg per 1 lb a.i./A)	Normalized dose** (µg a.i./bee per 1 lb a.i./A)
0	1	0.74	16	2.1
0	2	0.63	14	1.8
1	1	0.95	21	2.7
1	2	0.54	12	1.5
2	1	0.10	2.2	0.28
3	1	0.01	0.2	0.03

*The numerical values were not provided in the article. These values were estimated from Figure 4 of the article.
**Calculated by multiplying normalized dose by the weight of individual bee (*i.e.*, 0.128 g). Note that mg/kg is equivalent to µg/g.

In addition, cypermethrin residues were measured on flowers and leaves of the treated crop. On samples collected within one day after the application, residues were approximately 2 mg/kg on flowers and 1.5 mg/kg on leaves. This translates to 44 and 33 mg/kg per 1 lb a.i./A for flowers and leaves, respectively. As with the residues on bees, these data were extracted from a figure provided in the article (Figure 5), therefore, the residue values are approximate.

Dively and Kamel 2012

Dively and Kamel 2010 quantified levels of several insecticides in pumpkin (*Cucurbita pepo*) nectar after pesticides were applied to soil via chemigation. Pesticide residues in nectar are the result of systemic transport through pumpkin plants located in the treated soil. In 2009 and 2010, pumpkin plants located in the field were treated with imidacloprid, dinotefuran, thiamethoxam or oxamyl. Rates and application methods used for the different treatment plots are provided in **Tables 2-7** and **2-8**. There were 4 replicates for each application scenario and a control for each study year. Prior to blooming, paper bags were placed over flower buds. When the flowers bloomed, they were removed and their nectar was collected using syringes. Concentrations of the pesticides and their degradates were quantified using liquid chromatography-tandem mass spectrometry. Dively and Kamel 2012 reported concentrations of thiamethoxam, dinotefuran, imidacloprid and oxamyl in pollen collected during the same time as nectar in the study described above. Residues of thimethoxam, dinotefuran and imidacloprid were an order of magnitude above those reported in nectar. Oxamyl was not detected in pollen or nectar samples. Reported maximum concentrations of pesticides plus their degradates in nectar and pollen are provided in **Tables 2-7** and **2-8**, respectively.

Table 4-7. Pesticide concentrations measured in nectar.

Chemical*	Year	application type	Total application rate (lb/A)	Maximum concentration* (mg/kg)	Normalized concentration* (mg a.i./kg per 1 lb a.i./A)
Imidacloprid	2009	bedding drench	0.027	0.0007	0.026
Imidacloprid	2009	transplant	0.251	0.0113	0.045
Imidacloprid	2009	transplant	0.377	0.0178	0.047
Imidacloprid	2009	water/drip	0.377	0.0231	0.061
Dinotefruan	2009	transplant drip	0.270	0.0154	0.057
Thiamethoxam	2009	transplant	0.171	0.0186	0.109
dinotefuran	2009	Foliar spray	0.270	0.0216	0.0801
thiamethoxam	2009	Foliar spray	0.171	0.0124	0.0723
Imidacloprid	2010	bedding drench	0.027	ND	ND
Imidacloprid	2010	transplant	0.251	0.0067	0.027
Imidacloprid	2010	water/drip	0.377	0.016	0.042
Dinotefruan	2010	transplant drip	0.270	0.0109	0.040
Thiamethoxam	2010	transplant	0.171	0.0151	0.088
dinotefuran	2010	foliar spray	0.270	0.005	0.0185
dinotefuran	2010	foliar spray	0.270	0.016	0.0593
thiamethoxam	2010	foliar spray	0.171	0.0025	0.0146
thiamethoxam	2010	foliar spray	0.171	0.007	0.0408

*Represents parent and degradates

Table 4-8. Pesticide concentrations measured in pollen.

Chemical*	Year	application type	Total application rate (lb/A)	Maximum concentration* (mg/kg)	Normalized concentration* (mg a.i./kg per 1 lb a.i./A)
Imidacloprid	2009	bedding drench	0.027	0.0094	0.351
Imidacloprid	2009	transplant	0.251	0.0567	0.226
Imidacloprid	2009	transplant	0.377	0.1085	0.288
Imidacloprid	2009	water/drip	0.377	0.1285	0.341
Dinotefruan	2009	transplant drip	0.270	0.0812	0.301
Thiamethoxam	2009	transplant	0.171	0.1316	0.768
dinotefuran	2009	foliar spray	0.270	0.168	0.623
thiamethoxam	2009	foliar spray	0.171	0.162	0.946
Imidacloprid	2010	bedding drench	0.027	ND	ND
Imidacloprid	2010	transplant	0.251	0.0239	0.0953
Imidacloprid	2010	water/drip	0.377	0.0440	0.117
Dinotefruan	2010	transplant drip	0.270	0.0269	0.100
Thiamethoxam	2010	transplant	0.171	0.0420	0.245
dinotefuran	2010	foliar spray	0.270	0.0221	0.082
dinotefuran	2010	foliar spray	0.270	0.114	0.424
thiamethoxam	2010	foliar spray	0.171	0.0191	0.111
thiamethoxam	2010	foliar spray	0.171	0.0378	0.221

*Represents parent and degradates1

Fries and Wibran 1987

In this study, cypermethrin and an unnamed pyrethroid insecticide, referred to in the article as "PP 321" were applied to oilseed rape (*Brassica campestris*). On the day of the application, residues of the two insecticides were measured in pollen. Cypermethrin and PP321 were applied to one field via foliar spray at respective rates of 0.039 and 0.004 lb a.i./A (0.044 and 0.005 kg a.i./ha). Two honey bee colonies were placed at each field and pollen samples were collected from bees entering the hives using pollen traps. The reported concentrations of the pesticides in pollen (measured on the day of application), application rate normalized concentrations are provided in **Table 4-9**. One notable limitation of this study is a lack of replication; however, this study provides information that is useful for characterizing the concentrations of pesticides in pollen. The reported concentrations will be treated as maximum, rather than mean values.

Table 4-9. Reported concentrations of cypermethrin and PP321 in pollen.

Chemical	Application rate (lb a.i./A)	Reported concentration (mg/kg)	Normalized concentration (mg/kg per 1 lb a.i./A)
Cypermethrin	0.039	1.9	49
PP 321	0.004	0.2	50

Girolami *et al.* 2009

Girolami *et al.* 2009 quantified imidacloprid, clothianidin, thiamethoxam, and fipronil residues in guttation fluid. Guttation fluid was collected from corn seedlings grown in the field from seeds coated with imidacloprid (Gaucho® 0.5 mg a.i./seed), clothianidin (Poncho® 1.25 mg a.i./seed), thiamethoxam (Cruiser® 1.0 mg a.i./seed), and fipronil (Regent® 1.0 mg a.i./seed). Guttation fluid was collected in the field from 8-9 a.m. daily until a volume of 5 mL was reached, beginning at seedling emergence and continuing for the first 3 weeks after emergence. Concentrations of chemicals in guttation fluid were measured using high-performance liquid chromatography (HPLC). In corn, it was observed that guttation fluid can flow down the plant into the crown cup and persist throughout the day. Guttation fluid was observed until 9-10 a.m. throughout April and May. The reported mean measured concentrations for each chemical are reported in **Table 4-8**.

Table 4-8. Measured concentrations in guttation fluid (μg a.i./mL)

Clothianidin (1.25 mg ai/seed)	Imidacloprid (0.5mg ai/seed)	Thiamethoxam (1.0 mg ai/seed)
23.3	47	11.9

Hanny and Harvey 1982

The purpose of this study was to compare effects of two carbaryl formulations (Sevin® Sprayable and Sevin® XLR) on honey bees (*A. mellifera*) under field conditions. Two separate fields of corn (*Zea mays*; 40 A each) located in Wyoming were treated with a single application of 2.0 lb a.i./A carbaryl via aerial spray. Six honey bee colonies were located adjacent to each treated field. Dead bees were collected twice a day from 3 colonies adjacent to each treated field using "Todd dead bee traps." Pollen samples were collected from the other 3 colonies using "modified OAC pollen traps."

Reported mean concentrations of carbaryl on dead bees from the three colonies located adjacent to each site are provided in **Table 4-10**. These values were normalized to application rate and converted to

a dose basis by multiplying by the body weight of a worker bee (*i.e.,* 0.128 g). Pesticide residues on bees near fields treated with the Sevin® Sprayable formulation appear to be higher compared to the Sevin XLR formulation. Carbaryl residues were highest on bees found dead on the day of the application. For the Sevin® Sprayable Formulation, the mean concentration was 1.19 mg a.i./kg per 1 lb a.i./A, which corresponds to a dose of 0.152 μg a.i./bee per 1 lb a.i./A. For the Sevin® XLR Formulation, the mean concentration was 0.36 mg a.i./kg per 1 lb a.i./A, which corresponds to a dose of 0.152 μg a.i./bee per 1 lb a.i./A. One notable limitation of this study report is that it does not include standard deviations or maximum concentrations, which prevents understanding of the variability associated with the measured concentrations of carbaryl on bees.

Table 4-10. Mean number of dead bees, carbaryl concentrations (from application of Sevin® Sprayable and Sevin® XLR formulations) on dead bees collected from bee traps located adjacent to treated fields (from Hanny and Harvey 1982), and normalized to 1 lb a.i./A and to dose.

Day after application	Reported mean concentration of carbaryl on bees (mg/kg)		Normalized mean concentration on bees (mg/kg per 1 lb a.i./A)		Normalized dose (µg a.i./bee per 1 lb a.i./A)	
	Sevin Sprayable	Sevin XLR	Sevin Sprayable	Sevin XLR	Sevin Sprayable	Sevin XLR
0*	2.37	0.71	1.19	0.36	0.152	0.045
1	1.75	0.3	0.88	0.15	0.112	0.019
2	0.5	0.09	0.25	0.05	0.0320	0.0058
3	0.36	0.1	0.18	0.05	0.0230	0.0064
4	0.17	NA	0.09	NA	0.0109	NA
5	0.27	NA	0.14	NA	0.0173	NA
6	0.32	NA	0.16	NA	0.0205	NA

*represents day of treatment
NA = not available

Mean-measured concentrations (based on data from 3 colonies) of carbaryl in pollen are provided in **Tables 4-11** and **4-12**. In addition, this table provides the author reported proportions of each pollen sample that were represented by corn, which allows for normalization of reported concentrations of carbaryl in pollen samples to represent 100% corn. **Tables 4-11 and 4-12** also include concentrations of carbaryl normalized to 1 lb a.i./A and 100% corn pollen.

Table 4-11. Daily concentrations of carbaryl (from Sevin® Sprayable formulation) in pollen collected from bees foraging near corn fields treated with carbaryl and fraction of pollen represented by corn.

Day after application	Reported mean concentration of carbaryl in pollen (mg/kg)	Mean % of pollen represented by corn	Normalized concentration (mg/kg per 1 lb a.i./A based on 100% corn pollen)
0	1.9	30.5%	3.1
1	0.51	31.5%	0.080
2	1.22	26.2%	0.16
3	0.32	22.3%	0.036
4	0.15	62.3%	0.047
5	0.14	53.7%	0.038
6	0.14	49.3%	0.035

Table 4-12. Daily concentrations of carbaryl (From Sevin® XLR formulation) in pollen collected from bees foraging near corn fields treated with carbaryl and fraction of pollen represented by corn.

Day after application	Reported mean concentration of carbaryl in pollen (mg/kg)	Mean % of pollen represented by corn	Normalized concentration (mg/kg per 1 lb a.i./A based on 100% corn pollen)
0	0.95	30.5%	1.6
1	0.18	31.5%	0.028
2	0.08	26.2%	0.010
3	0.02	22.3%	0.002

Hoffman and Castle 2012

Hoffman and Castle 2012 quantified imidacloprid residues in guttation fluid from cantaloupes grown in fields. Imidacloprid was applied as a soil drip to the sixth node stage of young cantaloupe plants that had already begun to bloom at an application rate of 767.3 mL/ha. Four days after application, guttation fluid was observed and collected from 5 treated plants. The guttation fluid evaporated from the leaves of the cantaloupe by 8:00 a.m. Imidacloprid residues were detected at a mean concentration of 2.2 ± 0.55 μg a.i./L.

In a targeted monitoring study involving guttation fluid, cantaloupe were planted in the early fall (September-October), and imidacloprid was applied to young plants as a soil drench application at rates of 282 or 422 g a.i./ha (0.25 or 0.377 lbs a.i./A, respectively). Imidacloprid residues were measured, and

ranged from 0.04 to 37.4 μg a.i./mL. The highest concentration detected corresponds to the higher application rate (0.377 lbs a.i./A).

Koch and Weisser 1997

This study investigated potential contact based exposures of bees to pesticides through applications of a fluorescent tracer (sodium-fluorescein) to flowering apple (*Malus domestica*) orchards and *Phacelia tanacetifolia* (commonly referred to as *Phacelia*) fields located in Germany. From 1992-1997, nine trials (spraying/sampling events) were conducted in the orchards and five trails were conducted in the *Phacelia* fields. The sizes of the treated ground areas ranged 0.42-1.64 ha for the apple orchards and 0.25-1 ha for *Phacelia* fields. The tracer was applied at a rate of 20 g/10000 m^2 of vertical spray area for orchards and ground spray area for *Phacelia* fields. For orchards the ground based application rate ranged 0.018-0.020 lb a.i./A (average: 0.019) and for *Phacelia* fields, the rate was equivalent to 0.018 lbs a.i./A. The tests were conducted during the flowing period of the orchard and *Phacelia* fields, therefore the number of trials was limited to the duration of the flowering period. Following each application, bees were collected at closed hive entrances over a period of 20-30 min in 5 min intervals. Sample size varied per trial but was approximately 100 bees per sampling point (4316 bees were analyzed in the nine orchard trials; 1724 bees in the five *Phacelia* trials).

For the apple orchards, the mean measured amount of tracer per bee ranged 1.62-20.84 ng/bee for the nine trials, and an overall average of 6.33 ng/bee (**Table 4-13**). The range of averages is equivalent to 0.079-1.02 μg a.i./bee when normalized to 1 lb a.i./A and the normalized overall average is 0.33 μg a.i./bee. **Table 4-13** presents the mean residues measured for each trail on bees as reported by the authors and normalized to application rate. Values are normalized to mass pesticide per bee and mass chemical per kg of bee, the latter is calculated by dividing the mass of pesticide per bee by the weight of the bee, which is 0.128 g. In 8 of the 9 trials, the majority (>57%) of individual bees had ≤5 ng tracer (≤0.26 μg a.i./bee normalized to 1 lb a.i./A); however, for all but one of the trials, several bees had residues that exceeded 45 ng tracer (>2.4 μg a.i./bee normalized to 1 lb a.i./A). Of all the bees used to quantify levels of the tracer, approximately 3% (113 out of 4316) had levels that exceeded 45 ng tracer[20]. **Figure 4-1** presents the frequency distribution of measured residues on bees measured in the 9 trials with apple orchards.

[20] This was determined by first multiplying number of bees in each trial that exceeded 45 ng tracer by the number of bees in

Table 4-13. Mean residues measured residues on bees for apple orchard trials.

Trial	Number of bees	Mean deposit* (ng/bee)	Mass applied per ground area (lb/A)	Normalized mean deposit (µg a.i./bee per 1 lb a.i./A)	Normalized mean deposit (mg a.i./kg per 1 lb a.i./A)**
1	239	3.81	0.018	0.21	1.61
2	227	2.21	0.018	0.12	0.94
3	498	7.8	0.020	0.38	2.98
4	480	1.62	0.020	0.079	0.62
5	490	3.39	0.020	0.17	1.29
6	600	20.84	0.020	1.02	7.95
7	594	8.51	0.020	0.42	3.25
8	488	4.44	0.018	0.25	1.94
9	700	4.42	0.018	0.25	1.93
all	4316	6.33	0.019	0.33	2.54

*As reported by the authors.
**Calculated by dividing the normalized mean deposit per bee by the body weight of an adult worker bee, which is 0.128 g.

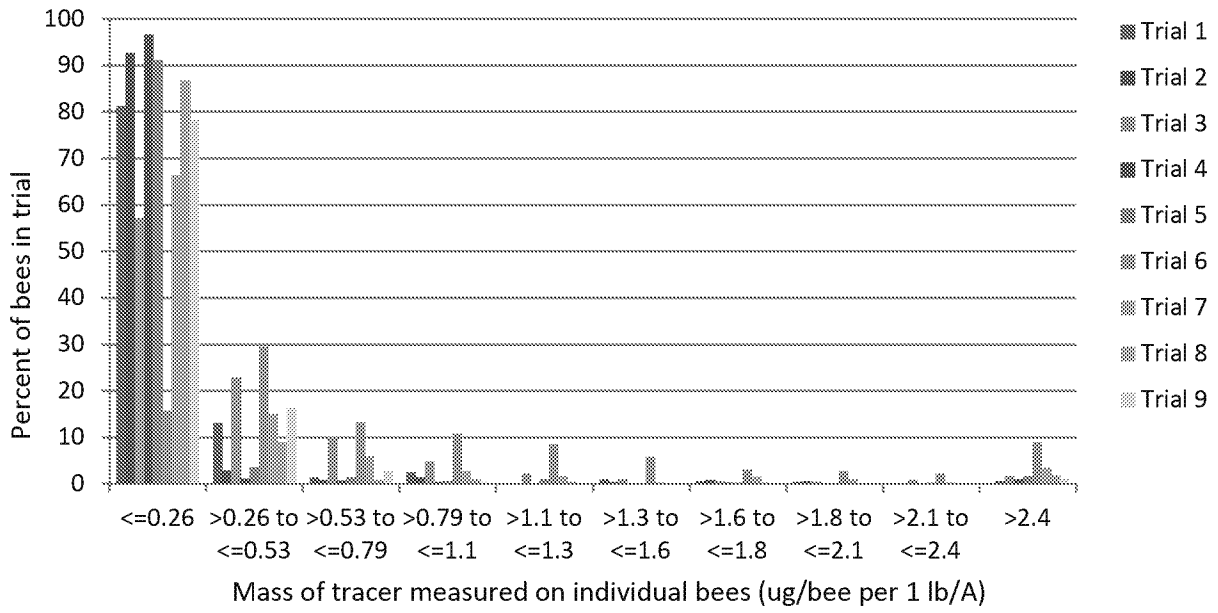

Figure 4-1. Frequency distribution of measured tracer on individual bees during 9 trials with apple orchards. Concentrations are normalized to application rate.

each trial to calculate the number of bees per trial that exceeded this level. The values for each trial were then added to estimate the total number of bees in the study exceeding 45 ng tracer.

For the *Phacelia* fields, the amount of tracer per bee was higher than those from the apple orchards, with mean measured values for the 5 trials ranging 6.34 to 35.77 ng/bee, and an overall average of 18.19 ng/bee. The range of averages is equivalent to 0.31 to 1.75 μg a.i./bee when normalized to 1 lb a.i./A and the normalized overall average is 0.89 μg a.i./bee. **Table 4-14** presents the mean residues measured for each trail on bees as reported by the authors and normalized to application rate. For the Phaceila trials, there were more bees with residues that exceeded 45 ng tracer (>2.4 µg a.i./bee normalized to 1 lb a.i./A) when compared to the apple trials. Of all the bees used to quantify levels of the tracer, approximately 14% (244 out of 1724) had levels that exceeded 45 ng tracer[21]. **Figure 4-2** presents the frequency distribution of measured residues on bees measured in the 5 trials with *Phacelia* fields. The highest residue detected in the Phacelia trials was 48 ng/bee, which is equivalent to 2.7 μg a.i./bee normalized to 1 lb a.i./A.

[21] This was determined by first multiplying number of bees in each trial that exceeded 45 ng tracer by the number of bees in each trial to calculate the number of bees per trial that exceeded this level. The values for each trial were then added to estimate the total number of bees in the study exceeding 45 ng tracer.

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Table 4-14. Mean residues measured residues on bees for apple orchard trials.

Trial	Number of bees	Mean deposit* (ng/bee)	Mass applied per ground area (lb/A)	Normalized mean deposit (µg a.i./bee per 1 lb a.i./A)	Normalized mean deposit (mg a.i./kg per 1 lb a.i./A)**
1	150	11.39	0.020	0.56	4.35
2	200	24.41	0.020	1.19	9.32
3	360	6.34	0.020	0.31	2.42
4	499	35.77	0.020	1.75	13.7
5	515	13.07	0.020	0.64	4.99
all	1724	18.19	0.020	0.89	6.94

*As reported by the authors.
**Calculated by dividing the normalized mean deposit per bee by the body weight of an adult worker bee, which is 0.128 g.

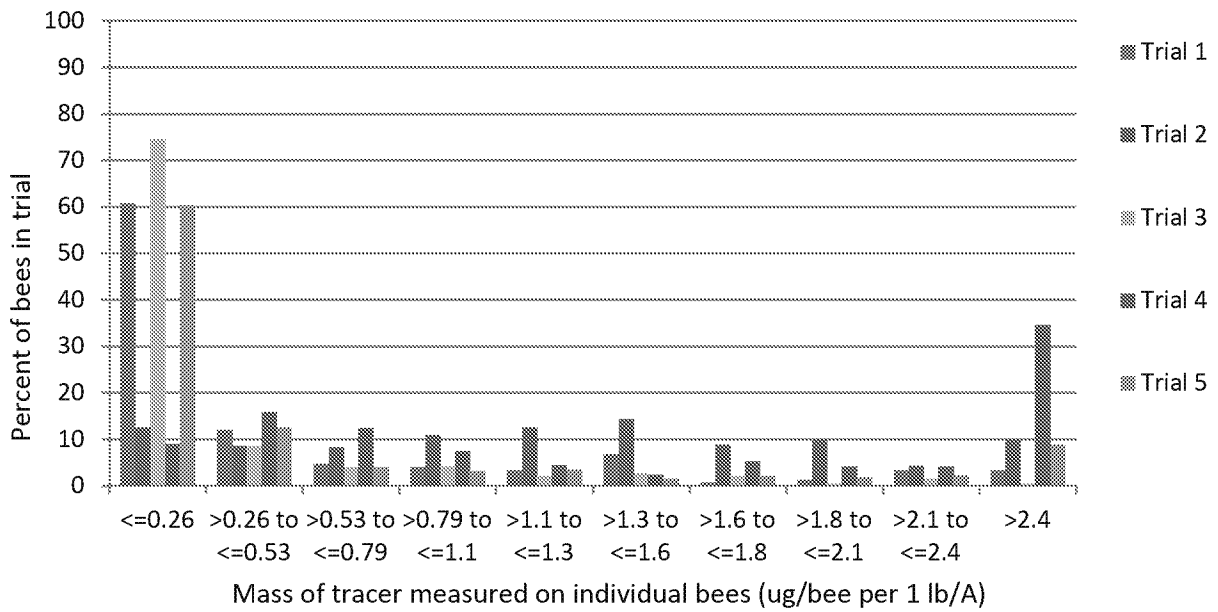

Figure 2-2. Frequency distribution of measured tracer on individual bees during 5 trials with *Phacelia* fields. Concentrations are normalized to application rate.

Lord *et al.* 1968

Lord *et al.* 1968 conducted a series of experiments that involved measurements of dimethoate or phorate in nectar of *Fuchsia sp.* and nasturtium (*Tropaeolum sp.*) plants after applications to soil. Plants were contained in 5 inch diameter pots (surface area = 0.013 m^2) located in a greenhouse. For both dimethoate and phorate, 25 mg a.i. were applied per pot, which is equivalent to a field application rate of 19 kg a.i./ha (or 17 lb a.i./A). According the Registration Eligibility Decision Documents for these

pesticides, this application rate is an order of magnitude higher than currently registered applications in the US.

In this experiment, pesticide concentrations were quantified in nectar 6 days after application because toxicity tests conducted with fruit flies (*Drosphila melanogaster*) over time established the highest mortality rates at this time point, suggesting that pesticide concentrations were at their peaks in nectar. Phorate was detected at approximately 0.100 µg a.i./mL, which was below the level where residues could be accurately quantified. Given the uncertainty associated with the phorate concentrations, these data will not be considered further. For dimethoate, mean concentrations in nectar were 0.741±0.259 µg a.i./mL for nasturtium and 2.890±0.550 µg a.i./mL for *Fuchsia*. These values were converted to a mass of pesticide per mass of nectar. First, these values were converted to a mass of pesticide per mass of sugar present in the nectar using the average of reported sugar contents of nectar from different plant species as reported by Wykes 1953 (*i.e.,* 240 mg sugar/mL nectar). The resulting values were divided by 2.5 mg nectar/mg sugar (Rortais *et al*. 2005) in order to translate the residues into a mass of pesticide per mass of nectar. These values were also normalized to 1 lb a.i./A by dividing by the application rate (**Table 4-15**).

Table 4-15. Dimethoate concentrations in nectar.

Plant species	Concentration			Normalized concentration (mg/kg per 1 lb a.i./A)
	(µg a.i./mL nectar)	(µg a.i./mg sugar)	(mg/kg nectar)	
nasturtium	0.741	0.00309	1.26	0.0726
Fuchsia	2.89	0.0120	4.82	0.283

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Reetz *et al.* 2011

Reetz *et al.* 2011 quantified clothianidin residues in guttation fluid collected from maize (corn) plants grown from seed under field conditions in Germany. Four different seed treatments were planted in April 2009; one seed treatment did not include clothianidin, and the remaining three seed treatments used one of two formulations of clothianidin (Poncho® (0.5 mg clothianidin a.i. /seed, equivalent to 0.006 or 0.067 lb a.i./A for seeding rates of 5321 and 60896 seeds/A, respectively)) or Poncho Pro® (1.25 mg clothianidin a.i./seed, equivalent to 0.015 or 0.168 lb a.i./A for seeding rates of 5321 and 60896 seeds/A, respectively)). Guttation fluid was collected May to July 2009, with sampling occurring every 2 days when weather conditions were good. Residue analysis was conducted using 50 µL of guttation fluid mixed with a 10 µL internal reference standard imidacloprid-d4. Concentrations were identified and quantified using a LC-HR-MS (LTQ™-Orbitrap spectrometer). Honeybees were observed collecting exuded fluids from *Potenilla* plants along the edge of the plot of seed treated maize (corn) plants when guttation fluid was being collected on the field, although none were observed on the field plot. Concentrations of 7.4 to 8 μg a.i./mL (equivalent to 47.6 to 110.4 μg a.i./mL, when normalized to 1 lb a.i./A) clothianidin were measured in the guttation fluid that was collected from maize plants. The greatest concentrations of clothianidin measured in guttation fluid occurred on the first day of sampling.

Škerl *et al.* 2009

This study involved applications of formulated products containing diazinon (Oleodiazinon), thiacloprid (Calypso SC 480) and difenoconazole (Score 250 EC) to apple (*Malus domestica*) orchards located in Slovenia in Spring of 2007. Application rates of the products were given as 15 L/ha Oleodiazinon, 0.2 L/ha Calypso SC 480 and 0.2 L/ha Score 250 EC. The application rates of the active ingredients were not included in the report; however, the mass of diazinon in Oleodiazinon (95 g/L) and the mass of thiacloprid in Calypso 480 SC (480 g/L) were both located on support documentation from the pesticide registrants' websites[22,23]. Therefore, the application rates of diazinon and thiacloprid used in this study were 1.3 and 0.09 lb a.i./A, respectively. The percent a.i. present in Score 250 EC was not located; therefore data for difenoconazole are not considered further. Pollen samples were collected using pollen traps located at two hives that were adjacent to a treated apple orchard. The concentrations of diazinon and thiacloprid in pollen are provided in **Table 4-16**. One notable limitation of this study is a

[22] Oleodiazinon: http://www.staehler.ch/pdf/tmb/oleodiazinons_d.pdf
[23] Calypso 480 SC: http://www.bayercropscience.com.au/resources/uploads/label/file7439.pdf

lack of replication; however, this study provides information that is useful for characterizing the concentrations of pesticides in pollen. The reported concentrations will be treated as maximum, rather than mean values.

Table 4-16. Diazinon and thiacloprid concentrations in pollen.

Chemical	Application rate (lb a.i./A)	Average Concentration (mg a.i./kg)	Normalized concentration (mg a.i./kg per 1 lb a.i./A)
Diazinon	1.3	1.98	1.5
Thiacloprid	0.09	0.09	1

Stoner and Eitzer 2012

The purpose of this study was to measure concentrations of imidacloprid and thiamethoxam in the pollen and nectar of squash (*Cucurbita pepo*) that was located in soil treated with these two pesticides. Applications were made via spray onto the soil or drip irrigation during 2009 and 2010 (on different fields). Application rates are provided in **Table 4-17**. Over the two years, the average application rates of imidacloprid and thiamethoxam were 0.344 and 0.127 lb a.i./A, respectively. The concentrations of imidacloprid and thiamethoxam in pollen and nectar were reported as a combination of both years and both application methods. The reported data are provided in **Table 4-18** and **Table 4-19**, respectively along with concentrations normalized to application rate.

Table 4-17. Application rates included in study conducted by Stoner and Eitzer (2012).

Chemical	Year	Application method	Application rate (lb a.i./A)	Application rate (kg a.i./ha)
Imidacloprid	2009	Soil spray (incorporation)	0.320	0.358
Imidacloprid	2009	Drip irrigation	0.320	0.358
Thiamethoxam	2009	Soil spray (incorporation)	0.125	0.140
Thiamethoxam	2009	Drip irrigation	0.125	0.140
Imidacloprid	2010	Soil spray (incorporation)	0.367	0.411
Imidacloprid	2010	Drip irrigation	0.367	0.411
Thiamethoxam	2010	Soil spray (incorporation)	0.128	0.143
Thiamethoxam	2010	Drip irrigation	0.128	0.143

Table 4-18. Reported and normalized concentrations of imidacloprid and thiamethoxam in pollen samples collected in 2009 and 2010.

Chemical	Average application rate (lb a.i./A)	Concentration (mg a.i./kg)			Number of samples	Normalized Concentration (mg a.i./kg per 1 lb a.i./A)		
		Mean (SD)	Min	Max		Mean (SD)	Min	Max
Imidacloprid	0.344	0.014 (0.008)	0.006	0.028	12	0.041 (0.023)	0.017	0.081
thiamethoxam	0.127	0.012 (0.009)	0.005	0.014	6	0.094 (0.071)	0.039	0.11

Table 4-19. Reported and normalized concentrations of imidacloprid and thiamethoxam in nectar samples collected in 2009 and 2010.

Chemical	Average application rate (lb a.i./A)	Concentration (mg a.i./kg)			Number of samples	Normalized Concentration (mg a.i./kg per 1 lb a.i./A)		
		Mean (SD)	Min	Max		Mean (SD)	Min	Max
Imidacloprid	0.344	0.010 (0.003)	0.005	0.014	6	0.029 (0.009)	0.015	0.041
thiamethoxam	0.127	0.011 (0.006)	0.005	0.020	6	0.087 (0.047)	0.039	0.16

Tapparo *et al.* 2011

Tapparo *et al.* 2011 quantified thiamethoxam, clothianidin, imidacloprid, and fipronil residues in guttation fluid from corn seedlings grown in both an open field and greenhouse experiments. Seeds were coated with thiamethoxam (Cruiser® 0.6 or 1.0 mg a.i./seed), clothianidin (Poncho® 1.25 mg a.i./seed), imidacloprid (Gaucho® 0.5, 1.0, or 1.25 mg a.i./seed), and fipronil (Regent® 0.5, 0.75, or 1.0 mg a.i./seed). Guttation fluid was collected every morning beginning the day after seedling emergence and continuing for 20 days after emergence. Residues of thiamethoxam, clothianidin, imidacloprid, and fipronil were analyzed using ultra high performance liquid chromatography (UHPLC). Measured fipronil concentrations were below the detection limit (LOD = 5.1 μg/L). In the greenhouse experiment, residues of thiamethoxam, clothianidin, and imidacloprid were the greatest on the first day after seedling emergence, decreasing over the first 10 days, and increased during the last 10 days of measurement (**Table 4-12**). Due to decrease in guttation fluid release with growth of the plant, concentration of the chemical may be highly concentrated within the limited water released by the plant. Also noted in a second greenhouse experiment was that the total concentrations of imidacloprid, clothianidin, and thiamethoxam measured in guttation fluid collected confirmed high peak concentrations occurring after seedling emergence (**Table 4-12**). In a third greenhouse experiment using thiamethoxam only, soil conditions (wetness) were used to measure the effects of chemical concentration within guttation fluid. The results indicated that under drier soil conditions the concentration of thiamethoxam is more concentrated (**Table 4-12**). During an open field experiment that was ran concurrently with the

greenhouse experiments, the measured concentrations of thiamethoxam, clothianidin, and imidacloprid also resulted in peaks in residue levels the first day after seedling emergence (**Table 4-12**).

Table 4-12. Measured concentrations in guttation fluid (µg a.i./mL)

Greenhouse Experiment 1			
Time	**Thiamethoxam (1.0 mg ai/seed) µg a.i./mL**	**Clothianidin (1.25 mg ai/seed) µg a.i./mL**	**Imidacloprid (0.5mg ai/seed) µg a.i./mL**
First day after SE	24.3	36.0	80.9
8-10 days after SE	3.6	8.8	17.3
11-20 days after SE	8.3	31.6	60.1

Greenhouse Experiment 2		
Thiamethoxam (1.0 mg ai/seed) µg a.i./mL	**Clothianidin (1.25 mg ai/seed) µg a.i./mL**	**Imidacloprid (1.25 mg ai/seed) µg a.i./mL**
146.0	101.7	345.8

Greenhouse Experiment 3 (Thiamethoxam only;1.0 mg a.i./seed)	
Conditions grown under	**Thiamethoxam µg a.i./mL**
wet (near saturation)	155
moderate	253
dry	1154

Field Experiment[1]			
Time	**Thiamethoxam (1.0 mg ai/seed) µg a.i./mL**	**Clothianidin (1.25 mg ai/seed) µg a.i./mL**	**Imidacloprid (0.5 mg ai/seed) µg a.i./mL**
First day after SE	227	46	222

SE = seedling emergence
[1] Assumptions made on mass a.i./seed

Wallner 2009

In this study, boscalid and prothioconazol were sprayed onto an oilseed rape (*Brassica sp.*) field at rates of 0.45 and 0.22 lb a.i./A (0.500 and 0.250 kg a.i./ha), respectively. In addition, the seeds were treated with clothianidin. Honey bees from two apiaries located adjacent to the treated field were used to collect pollen and nectar. Samples were collected over a 7 day period, including the day before the application, the day of the application, and the following 5 days. Prothioconazol and clothianidin were not detected in the pollen collected by the honey bees. Boscalid was detected in all pollen samples. The average boscalid concentration in pollen was 13.9 mg/kg on the day of the application and 26.2 mg/kg on the day after application (**Table 4-20**). All three pesticides were detected in nectar collected from

bees. On the day of the application, boscalid and prothioconazol were detected at 1.43 and 0.69 mg/kg, respectively (**Tables 4-21** and **4-22**). Clothianidin was detected at a maximum of 0.003 mg/kg.

Table 4-20. Boscalid concentrations in pollen.

Day	Average Concentration (mg a.i./kg)	Normalized concentration (mg a.i./kg per 1 lb a.i./A)
0	13.9	31
1	26.2	58
2-5	4.7	10

Table 4-21. Boscalid concentrations in nectar.

Day	Average Concentration (mg a.i./kg)	Normalized concentration (mg a.i./kg per 1 lb a.i./A)
0	1.43	3.2
1	0.13	0.29
2	0.017	0.038

Table 4-22. Prothioconazol concentrations in nectar.

Day	Average Concentration (mg a.i./kg)	Normalized concentration (mg a.i./kg per 1 lb a.i./A)
0	0.69	3.2
1	0.06	0.27
2	0.017	0.077

Appendix 5. Transpiration Stream Concentration Factors (TSCFs)

Median and 95th percentile estimates of TSCF values calculated by EPA using empirical data reported by Briggs 1982 and **1983.**

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
-1		0.060662	0.351114
-0.99		0.061637	0.352286
-0.98		0.062624	0.353473
-0.97		0.063624	0.354673
-0.96		0.064635	0.355888
-0.95		0.06566	0.357116
-0.94		0.066696	0.358358
-0.93		0.067746	0.359614
-0.92		0.068807	0.360885
-0.91		0.069882	0.362169
-0.9		0.070969	0.363467
-0.89		0.072069	0.36478
-0.88		0.073182	0.366106
-0.87		0.074308	0.367447
-0.86		0.075447	0.368802
-0.85		0.076599	0.370171
-0.84		0.077765	0.371554
-0.83		0.078943	0.372951
-0.82		0.080135	0.374363
-0.81		0.08134	0.375789
-0.8		0.082559	0.377229
-0.79		0.083791	0.378683
-0.78		0.085037	0.380152
-0.77		0.086297	0.381634
-0.76		0.08757	0.383131
-0.75		0.088857	0.384642
-0.74		0.090158	0.386167
-0.73		0.091472	0.387707
-0.72		0.092801	0.38926
-0.71		0.094143	0.390828
-0.7		0.0955	0.392409
-0.69		0.09687	0.394005
-0.68		0.098255	0.395614
-0.67		0.099654	0.397238
-0.66		0.101067	0.398876
-0.65		0.102494	0.400527
-0.64		0.103936	0.402193
-0.63		0.105391	0.403872
-0.62		0.106862	0.405565

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Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
-0.61		0.108346	0.407272
-0.6		0.109845	0.408992
-0.59		0.111358	0.410726
-0.58		0.112886	0.412473
-0.57	0.19	0.114429	0.414235
-0.57		0.114429	0.414235
-0.56		0.115985	0.416009
-0.55		0.117557	0.417797
-0.54		0.119143	0.419598
-0.53		0.120743	0.421412
-0.52		0.122358	0.42324
-0.51		0.123988	0.425081
-0.5		0.125632	0.426934
-0.49		0.127291	0.428801
-0.48		0.128964	0.43068
-0.47	0.21	0.130652	0.432572
-0.47		0.130652	0.432572
-0.46		0.132355	0.434477
-0.45		0.134072	0.436395
-0.44		0.135804	0.438325
-0.43		0.13755	0.440267
-0.42		0.139311	0.442222
-0.41		0.141087	0.444189
-0.4		0.142877	0.446168
-0.39		0.144681	0.448158
-0.38		0.1465	0.450161
-0.37		0.148334	0.452176
-0.36		0.150182	0.454202
-0.35		0.152044	0.45624
-0.34		0.153921	0.45829
-0.33		0.155812	0.46035
-0.32		0.157718	0.462422
-0.31		0.159637	0.464506
-0.3		0.161571	0.4666
-0.29		0.163519	0.468705
-0.28		0.165482	0.47082
-0.27		0.167458	0.472947
-0.26		0.169448	0.475084
-0.25		0.171452	0.477231
-0.24		0.173471	0.479389
-0.23		0.175502	0.481556
-0.22		0.177548	0.483734
-0.21		0.179607	0.485922
-0.2		0.18168	0.488119
-0.19		0.183767	0.490326
-0.18		0.185867	0.492542
-0.17		0.18798	0.494768

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
-0.16		0.190107	0.497003
-0.15		0.192246	0.499247
-0.14		0.194399	0.5015
-0.13	0.28	0.196565	0.503762
-0.13		0.196565	0.503762
-0.12	0.051	0.198744	0.506032
-0.12		0.198744	0.506032
-0.11		0.200935	0.508311
-0.1		0.203139	0.510598
-0.09		0.205356	0.512894
-0.08		0.207585	0.515197
-0.07		0.209826	0.517509
-0.06		0.21208	0.519828
-0.05		0.214345	0.522155
-0.04		0.216623	0.524489
-0.03		0.218912	0.526831
-0.02		0.221213	0.52918
-0.01		0.223526	0.531536
7.53E-16		0.22585	0.533899
0.01		0.228185	0.536268
0.02		0.230531	0.538645
0.03		0.232889	0.541027
0.04		0.235257	0.543416
0.05		0.237635	0.545811
0.06		0.240024	0.548213
0.07		0.242424	0.55062
0.08		0.244833	0.553032
0.09		0.247253	0.555451
0.1		0.249682	0.557875
0.11		0.252121	0.560304
0.12		0.254569	0.562738
0.13		0.257027	0.565177
0.14		0.259493	0.567621
0.15		0.261969	0.570069
0.16		0.264453	0.572522
0.17		0.266945	0.574979
0.18		0.269446	0.577441
0.19		0.271955	0.579906
0.2		0.274471	0.582376
0.21		0.276995	0.584849
0.22		0.279527	0.587326
0.23		0.282065	0.589806
0.24		0.284611	0.592289
0.25		0.287163	0.594776
0.26		0.289722	0.597265
0.27		0.292287	0.599757
0.28		0.294859	0.602252

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
0.29		0.297435	0.604749
0.3		0.300018	0.607249
0.31		0.302606	0.609751
0.32		0.305198	0.612255
0.33		0.307796	0.61476
0.34		0.310398	0.617268
0.35		0.313004	0.619777
0.36		0.315615	0.622287
0.37		0.318229	0.624798
0.38		0.320846	0.627311
0.39		0.323467	0.629824
0.4		0.326091	0.632338
0.41		0.328717	0.634853
0.42		0.331346	0.637368
0.43		0.333977	0.639883
0.44		0.33661	0.642398
0.45		0.339244	0.644913
0.46		0.34188	0.647428
0.47		0.344516	0.649942
0.48		0.347154	0.652456
0.49		0.349791	0.654969
0.5		0.352429	0.657481
0.51		0.355067	0.659991
0.52		0.357704	0.662501
0.53		0.36034	0.665008
0.54		0.362975	0.667514
0.55		0.365608	0.670018
0.56		0.36824	0.67252
0.57		0.37087	0.67502
0.58		0.373498	0.677517
0.59		0.376122	0.680011
0.6		0.378744	0.682503
0.61		0.381363	0.684991
0.62		0.383978	0.687477
0.63		0.386588	0.689958
0.64		0.389195	0.692436
0.65		0.391797	0.69491
0.66		0.394394	0.69738
0.67		0.396986	0.699846
0.68		0.399572	0.702307
0.69		0.402152	0.704763
0.7		0.404727	0.707214
0.71		0.407294	0.70966
0.72		0.409855	0.712101
0.73		0.412408	0.714535
0.74		0.414954	0.716964
0.75		0.417491	0.719387

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
0.76		0.420021	0.721803
0.77		0.422542	0.724212
0.78		0.425054	0.726615
0.79		0.427557	0.72901
0.8	0.47	0.43005	0.731398
0.8		0.43005	0.731398
0.81		0.432533	0.733778
0.82		0.435006	0.736151
0.83		0.437468	0.738514
0.84		0.439919	0.74087
0.85		0.442359	0.743216
0.86		0.444787	0.745554
0.87		0.447204	0.747882
0.88		0.449608	0.750201
0.89		0.451999	0.752509
0.9		0.454377	0.754807
0.91		0.456742	0.757095
0.92		0.459094	0.759372
0.93		0.461431	0.761638
0.94		0.463754	0.763892
0.95		0.466062	0.766135
0.96		0.468355	0.768365
0.97		0.470633	0.770583
0.98		0.472895	0.772788
0.99		0.475142	0.774981
1		0.477371	0.77716
1.01		0.479584	0.779325
1.02		0.48178	0.781476
1.03		0.483959	0.783612
1.04	0.47	0.48612	0.785734
1.04		0.48612	0.785734
1.05		0.488263	0.787841
1.06		0.490388	0.789933
1.07		0.492494	0.792008
1.08	0.54	0.494581	0.794068
1.08		0.494581	0.794068
1.09		0.496649	0.796111
1.1		0.498697	0.798137
1.11		0.500726	0.800147
1.12		0.502734	0.802138
1.13		0.504722	0.804112
1.14		0.506689	0.806067
1.15		0.508634	0.808004
1.16		0.510559	0.809922
1.17		0.512461	0.811821
1.18		0.514342	0.8137
1.19		0.5162	0.815558

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
1.2		0.518035	0.817397
1.21		0.519848	0.819214
1.22		0.521637	0.821011
1.23		0.523403	0.822786
1.24		0.525146	0.824539
1.25		0.526864	0.82627
1.26		0.528558	0.827978
1.27		0.530227	0.829663
1.28		0.531872	0.831325
1.29		0.533491	0.832963
1.3		0.535085	0.834578
1.31		0.536653	0.836168
1.32		0.538196	0.837733
1.33		0.539712	0.839273
1.34		0.541202	0.840788
1.35		0.542666	0.842277
1.36		0.544102	0.843739
1.37		0.545512	0.845176
1.38		0.546894	0.846586
1.39		0.548249	0.847968
1.4		0.549576	0.849323
1.41		0.550875	0.850651
1.42		0.552145	0.85195
1.43		0.553388	0.853222
1.44		0.554601	0.854464
1.45		0.555786	0.855678
1.46		0.556942	0.856862
1.47		0.558069	0.858017
1.48		0.559166	0.859142
1.49	0.67	0.560234	0.860237
1.49		0.560234	0.860237
1.5		0.561272	0.861301
1.51		0.56228	0.862335
1.52		0.563259	0.863339
1.53		0.564206	0.864311
1.54		0.565124	0.865251
1.55		0.566011	0.86616
1.56		0.566867	0.867038
1.57	0.22	0.567692	0.867883
1.57		0.567692	0.867883
1.58		0.568487	0.868696
1.59		0.56925	0.869477
1.6		0.569982	0.870225
1.61		0.570682	0.87094
1.62		0.571352	0.871622
1.63		0.571989	0.872271
1.64		0.572595	0.872887

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
1.65		0.573169	0.873469
1.66		0.573711	0.874018
1.67		0.574221	0.874533
1.68		0.574699	0.875014
1.69		0.575145	0.875461
1.7		0.575559	0.875874
1.71		0.57594	0.876253
1.72		0.576289	0.876597
1.73		0.576606	0.876907
1.74		0.57689	0.877183
1.75		0.577141	0.877424
1.76		0.57736	0.877631
1.77		0.577546	0.877803
1.78		0.5777	0.87794
1.79		0.577821	0.878043
1.8	0.5	0.577909	0.878111
1.8		0.577909	0.878111
1.81		0.577965	0.878145
1.82		0.577988	0.878144
1.83		0.577978	0.878108
1.84		0.577935	0.878038
1.85		0.57786	0.877933
1.86		0.577752	0.877794
1.87		0.577611	0.877621
1.88		0.577438	0.877413
1.89		0.577232	0.87717
1.9		0.576994	0.876894
1.91		0.576723	0.876583
1.92		0.576419	0.876239
1.93		0.576083	0.87586
1.94		0.575714	0.875448
1.95		0.575314	0.875002
1.96		0.574881	0.874523
1.97		0.574415	0.87401
1.98	0.55	0.573918	0.873465
1.98		0.573918	0.873465
1.99		0.573389	0.872886
2		0.572827	0.872274
2.01		0.572234	0.87163
2.02		0.571609	0.870953
2.03		0.570952	0.870244
2.04		0.570264	0.869503
2.05		0.569545	0.86873
2.06		0.568794	0.867926
2.07		0.568012	0.86709
2.08		0.567199	0.866223
2.09		0.566355	0.865326

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
2.1		0.56548	0.864397
2.11		0.564575	0.863438
2.12		0.563639	0.86245
2.13		0.562673	0.861431
2.14		0.561677	0.860383
2.15		0.560651	0.859305
2.16		0.559595	0.858199
2.17		0.558509	0.857063
2.18		0.557394	0.8559
2.19		0.55625	0.854708
2.2		0.555076	0.853489
2.21		0.553874	0.852242
2.22		0.552643	0.850968
2.23		0.551384	0.849667
2.24		0.550096	0.84834
2.25		0.54878	0.846986
2.26		0.547437	0.845607
2.27		0.546065	0.844202
2.27	0.94	0.546065	0.844202
2.28		0.544667	0.842772
2.29		0.543241	0.841318
2.3		0.541788	0.839838
2.31		0.540308	0.838335
2.32		0.538802	0.836808
2.33		0.53727	0.835257
2.34		0.535712	0.833683
2.35		0.534128	0.832087
2.36		0.532519	0.830468
2.37		0.530884	0.828827
2.38		0.529225	0.827165
2.39		0.527541	0.825481
2.4		0.525832	0.823776
2.41		0.524099	0.82205
2.42		0.522343	0.820305
2.43		0.520563	0.818539
2.44		0.518759	0.816754
2.45		0.516933	0.814949
2.46		0.515084	0.813126
2.47		0.513212	0.811284
2.48		0.511318	0.809424
2.49		0.509402	0.807546
2.5		0.507465	0.80565
2.51		0.505507	0.803737
2.52		0.503527	0.801808
2.53		0.501527	0.799862
2.54		0.499507	0.7979
2.55		0.497467	0.795922

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
2.56		0.495406	0.793928
2.57		0.493327	0.791919
2.58		0.491228	0.789896
2.59		0.489111	0.787858
2.6		0.486975	0.785805
2.61		0.484821	0.783739
2.62		0.482649	0.781659
2.63		0.48046	0.779566
2.64		0.478254	0.777459
2.64	0.37	0.478254	0.777459
2.65		0.476031	0.77534
2.66		0.473791	0.773209
2.67		0.471535	0.771065
2.68		0.469264	0.76891
2.69		0.466977	0.766743
2.7		0.464674	0.764565
2.71		0.462357	0.762375
2.72		0.460025	0.760175
2.73		0.45768	0.757965
2.74		0.45532	0.755744
2.75		0.452947	0.753513
2.76		0.450561	0.751273
2.77		0.448162	0.749023
2.78		0.44575	0.746764
2.79		0.443327	0.744496
2.8		0.440891	0.742219
2.8	0.47	0.440891	0.742219
2.81		0.438445	0.739933
2.82		0.435987	0.73764
2.83		0.433518	0.735338
2.84		0.431039	0.733029
2.85		0.42855	0.730712
2.86		0.426051	0.728387
2.87		0.423542	0.726056
2.88		0.421025	0.723717
2.89		0.418499	0.721372
2.89	0.51	0.418499	0.721372
2.9		0.415964	0.71902
2.91		0.413422	0.716662
2.92		0.410871	0.714298
2.93		0.408314	0.711928
2.94		0.405749	0.709552
2.95		0.403177	0.707171
2.96		0.4006	0.704784
2.97		0.398016	0.702392
2.98		0.395426	0.699995
2.99		0.392831	0.697593

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
3		0.390231	0.695187
3.01		0.387626	0.692776
3.02		0.385017	0.690361
3.03		0.382404	0.687942
3.04		0.379787	0.685519
3.05		0.377166	0.683092
3.06		0.374542	0.680661
3.07		0.371916	0.678227
3.08		0.369287	0.67579
3.09		0.366656	0.67335
3.1		0.364023	0.670907
3.11		0.361388	0.66846
3.12		0.358753	0.666012
3.12	0.26	0.358753	0.666012
3.13		0.356116	0.663561
3.14		0.353479	0.661107
3.15		0.350841	0.658652
3.16		0.348203	0.656194
3.17		0.345566	0.653735
3.18		0.342929	0.651273
3.19		0.340293	0.648811
3.2		0.337658	0.646347
3.21		0.335025	0.643881
3.22		0.332393	0.641415
3.23		0.329763	0.638948
3.24		0.327136	0.63648
3.25		0.324511	0.634011
3.26		0.321889	0.631542
3.27		0.31927	0.629072
3.28		0.316654	0.626602
3.29		0.314042	0.624133
3.3		0.311434	0.621663
3.31		0.308831	0.619194
3.32		0.306231	0.616725
3.33		0.303637	0.614257
3.34		0.301047	0.611789
3.35		0.298462	0.609323
3.36		0.295883	0.606857
3.37		0.29331	0.604393
3.38		0.290742	0.60193
3.39		0.288181	0.599468
3.4		0.285626	0.597008
3.41		0.283078	0.59455
3.42		0.280536	0.592094
3.43		0.278002	0.58964
3.44		0.275475	0.587188
3.45		0.272955	0.584739

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
3.46		0.270443	0.582293
3.47		0.267939	0.579849
3.48		0.265443	0.577408
3.49		0.262956	0.57497
3.5		0.260477	0.572535
3.51		0.258007	0.570104
3.52		0.255546	0.567676
3.53		0.253094	0.565252
3.54		0.250651	0.562831
3.55		0.248218	0.560415
3.56		0.245795	0.558003
3.57		0.243381	0.555596
3.58		0.240978	0.553192
3.59		0.238585	0.550794
3.6		0.236202	0.5484
3.61		0.23383	0.546011
3.62		0.231468	0.543628
3.63		0.229117	0.54125
3.64		0.226778	0.538877
3.65		0.224449	0.53651
3.66		0.222132	0.534148
3.67		0.219826	0.531793
3.68		0.217532	0.529444
3.69		0.21525	0.527101
3.7		0.21298	0.524764
3.7	0.11	0.21298	0.524764
3.71		0.210721	0.522434
3.72		0.208475	0.520111
3.73		0.206241	0.517795
3.74		0.20402	0.515486
3.75		0.20181	0.513184
3.76		0.199614	0.51089
3.77		0.19743	0.508603
3.78		0.195259	0.506324
3.79		0.193101	0.504052
3.8		0.190956	0.501789
3.81		0.188825	0.499534
3.82		0.186706	0.497288
3.83		0.184601	0.495049
3.84		0.182509	0.49282
3.85		0.180431	0.490599
3.86		0.178366	0.488388
3.87		0.176315	0.486185
3.88		0.174277	0.483992
3.89		0.172254	0.481808
3.9		0.170244	0.479634
3.91		0.168248	0.477469

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
3.92		0.166266	0.475314
3.93		0.164299	0.473169
3.94		0.162345	0.471035
3.95		0.160405	0.468911
3.96		0.15848	0.466797
3.97		0.156569	0.464693
3.98		0.154672	0.462601
3.99		0.152789	0.460519
4		0.150921	0.458448
4.01		0.149068	0.456389
4.02		0.147228	0.45434
4.03		0.145403	0.452303
4.04		0.143593	0.450277
4.05		0.141797	0.448263
4.06		0.140016	0.446261
4.07		0.138249	0.44427
4.08		0.136497	0.442292
4.09		0.134759	0.440325
4.1		0.133036	0.438371
4.11		0.131328	0.436429
4.12		0.129634	0.434499
4.13		0.127955	0.432582
4.14		0.12629	0.430678
4.15		0.12464	0.428786
4.16		0.123005	0.426907
4.17		0.121384	0.42504
4.18		0.119778	0.423187
4.19		0.118186	0.421347
4.2		0.116609	0.41952
4.21		0.115046	0.417707
4.22		0.113498	0.415906
4.23		0.111965	0.414119
4.24		0.110446	0.412346
4.25		0.108941	0.410586
4.26		0.107451	0.408839
4.27		0.105975	0.407107
4.28		0.104513	0.405388
4.29		0.103066	0.403683
4.3		0.101633	0.401992
4.31		0.100214	0.400314
4.32		0.09881	0.398651
4.33		0.09742	0.397002
4.34		0.096043	0.395367
4.35		0.094681	0.393746
4.36		0.093333	0.392139
4.37		0.091999	0.390546
4.38		0.090679	0.388968

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
4.39		0.089373	0.387403
4.4		0.08808	0.385854
4.41		0.086802	0.384318
4.42		0.085537	0.382797
4.43		0.084285	0.38129
4.44		0.083048	0.379797
4.45		0.081824	0.378319
4.46		0.080613	0.376856
4.47		0.079416	0.375406
4.48		0.078232	0.373971
4.49		0.077061	0.372551
4.5		0.075904	0.371145
4.51		0.07476	0.369753
4.52		0.073629	0.368376
4.53		0.07251	0.367013
4.54		0.071405	0.365665
4.55		0.070313	0.364331
4.56		0.069233	0.363011
4.57		0.068166	0.361706
4.58		0.067112	0.360415
4.59		0.066071	0.359138
4.6		0.065042	0.357876
4.6	0.06	0.065042	0.357876
4.61		0.064025	0.356627
4.62		0.06302	0.355393
4.63		0.062028	0.354173
4.64		0.061048	0.352967
4.65		0.06008	0.351776
4.66		0.059124	0.350598
4.67		0.05818	0.349434
4.68		0.057248	0.348284
4.69		0.056328	0.347148
4.7		0.055419	0.346026
4.71		0.054522	0.344918
4.72		0.053636	0.343823
4.73		0.052762	0.342742
4.74		0.051899	0.341675
4.75		0.051047	0.340621
4.76		0.050206	0.33958
4.77		0.049377	0.338553
4.78		0.048558	0.337539
4.79		0.04775	0.336539
4.8		0.046953	0.335551
4.81		0.046167	0.334577
4.82		0.045392	0.333616
4.83		0.044626	0.332667
4.84		0.043872	0.331732

Log K_{ow}	Empirical TSCFs (Briggs 1982)	Median TSCF estimate	Upper 95th percentile TSCF estimate
4.85		0.043127	0.330809
4.86		0.042393	0.329899
4.87		0.041669	0.329001
4.88		0.040955	0.328116
4.89		0.040251	0.327244
4.9		0.039557	0.326383
4.91		0.038872	0.325535
4.92		0.038197	0.324699
4.93		0.037532	0.323875
4.94		0.036877	0.323063
4.95		0.03623	0.322263
4.96		0.035593	0.321475
4.97		0.034966	0.320698
4.98		0.034347	0.319933
4.99		0.033737	0.319179
5		0.033137	0.318436

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