Pesticide Risk Assessment for Pollinators: Summary of a SETAC Pellston Workshop

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Summary of the SETAC Pellston Workshop on Pesticide Risk Assessment for Pollinators
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Worldwide declines in native and managed pollinators have led to an increased global dialogue and focus concerning the potential factors that may be causing these declines. Although a number of factors have been hypothesized as potential contributors to pollinator declines, at this time, no single factor has been identified as the cause. The available science suggests that pollinator declines are a result of multiple factors that may be acting in various combinations. Research is being directed at identifying the individual and combined stressors that are most strongly associated with pollinator declines. Pesticide use is one of the factors under consideration.

In an effort to further the global dialogue, the Society of Environmental Toxicology and Chemistry (SETAC) held a Pellston Workshop\(^1\) to explore the state of the science on pesticide risk assessment for pollinators. The proposal for this SETAC workshop was developed by a steering committee comprised of members from government, business, academic, and nongovernmental organizations interested in advancing the science to understand the effect of pesticides on nontarget insects. Workshop participants were tasked to advance the current state of the science of pesticide risk assessment by more thoroughly vetting quantitative and qualitative measures of exposure and effects on the individual bee and on the colony. In doing so, the participants aimed to synthesize the global understanding and work that has taken place, to move toward a harmonized process for evaluating and quantitatively characterizing risk to pollinators from exposure to pesticides, and to identify the data needed to inform that process.

The SETAC Pellston Workshop on Pesticide Risk Assessment for Pollinators focused on four major goals:

1) design or identify testing protocols to estimate potential exposure of bees to pesticide residues in pollen, nectar, and other exposure routes;

2) design or identify testing protocols to measure effects of pesticides on developing brood and adult honey bees at both the individual and the colony levels;

3) propose a tiered approach for characterizing the potential risk of pesticides to pollinators; and

4) explore the applicability of testing protocols used for honey (Apis) bees to measure effects of pesticides and pesticide risk on native (non-Apis) bee species.

\(^1\) The first SETAC Pellston Workshop was held in 1977 to address the needs and means for assessing the hazards of chemicals to aquatic life. Since then, many such workshops 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 for their technical basis and their comprehensive, state-of-the-science reviews.
Although the term “pollinators” encompasses a broad number of taxa, for the purposes of this SETAC workshop and its proceedings, the term “pollinators” refers specifically to subspecies and strains of *Apis mellifera* that originated in Europe (i.e., the honey bee) and other, non-*Apis mellifera* bees. The workshop built upon the numerous efforts of different organizations, regulatory authorities, and individuals, both nationally and internationally, aiming to better understand the role and effects of pesticide products on native and honey bees.\(^2\)

Similar to other timely and relevant scientific issues addressed by SETAC Pellston Workshops, the issue of pollinator protection is of high interest to scientists employed by governments, business, academia, and nongovernmental organizations. For this reason, SETAC requires that its workshops be similarly balanced. The Pellston Workshop on Pesticide Risk Assessment for Pollinators represented an exceptionally diverse composition by both employer sector and geography. The 48 participants (35 panelists and 13 Steering Committee members) included individuals from business, nongovernmental organizations, federal and state governments, beekeepers, and academia and represented five continents (South America, Europe, Australia, North America, and Africa).

### 1 Overview of Honey Bee Biology

A key goal of regulatory authorities is to protect nontarget organisms from potential adverse effects of pesticides. Because it is not possible to test all species, the pesticide risk assessment framework relies on surrogate species to represent major taxa, including insect pollinators. The European honey bee (*A. mellifera*), among the many different bee species, is a desirable surrogate test species because it is both commercially valued and adaptable to laboratory research. The honey bee provides pollination services for agriculture, provides various hive products (e.g., honey, wax), and can survive under laboratory and research conditions, allowing us to understand how it responds to pesticides. In many countries, such as Canada and the United States, the honey bee is used as a surrogate for many other nontarget terrestrial insects and for insect pollinators. While honey bees frequently are subject to collateral effects from the use of pesticides in crop production, they also are the beneficiaries of pesticide applications that beekeepers routinely employ to handle pest problems that occur in managed hives. The in-hive use of pesticides by beekeepers and the potential exposure of bees to environmental mixtures of pesticides used in agriculture, coupled with the complex social organization and biology of bees, can complicate pesticide risk assessment. Therefore, it is important to understand the

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ecology and biology of both the surrogate test organism and those species it is intended to protect.

From a risk assessment perspective, several aspects of honey bee biology are important to consider because they potentially impact the studies required as well as the approach for evaluating risks. Colony growth and survival depend on the collective actions of individuals who perform various critical tasks; therefore, honey bee colonies act collectively as a “superorganism.” Different castes of bees within the hive structure have different functions, which can result in differential exposure in terms of duration, magnitude, and mode (direct versus indirect, secondary exposure). The survival of an individual bee may be of little consequence because colonies typically have a 10% to 30% reserve of workers, which reflects and accommodates the high turnover rate of the individual and the flexibility of the colony to adapt to its environment. A description of the roles of various castes within the hive and the implication for risk assessments follows.

A honey bee colony is made up of one queen, several drones, thousands of workers, and many immature bees in various stages of development (eggs, larvae, pupae). Worker bees are sexually undeveloped females and constitute the vast majority of the adults in a colony. All of the work inside and outside the colony is done by worker bees. Older workers forage outside the hive for pollen and nectar, and thus are vulnerable to contact exposure to pesticides during foraging as well as dietary exposure during collection or ingestion of pollen and nectar. Workers also serve as a vector for bringing contaminants back to the hive. Young workers clean cells and attend brood, whereas middle-aged workers do a variety of tasks mainly within the hive. Both young and middle-aged workers can have secondary exposure to pesticides through contaminated food brought back to the hive.

Each colony has a single queen. Once she mates with drones, the queen returns to the hive to begin the task of egg-laying; she will lay up to 1200 eggs per day for several years. The queen performs no other work in the hive, and she is fed royal jelly throughout her lifespan. Drones are male bees whose sole function in the hive is to serve as sperm donors for new queens. Like younger and middle-aged workers, queens and drones can have secondary exposure to pesticides through contaminated food that is brought back to the hive or through intentional use in the colony by beekeepers.

Inputs by worker bees into the colony include pollen, nectar, water, and plant exudates (e.g., sap) used to make propolis. Pollen is used as the source of protein. It may be consumed directly, consumed and used to produce brood food or royal jelly, or stored and consumed later. While larval bees may consume

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3 Brood food is produced by nurse bees and is used to feed developing larval bees; the clear component is secreted from the hypopharyngeal glands and is mixed with a milky component from the mandibular gland. These secretions provide a source of both protein and carbohydrate to developing brood (Winston ML. 1991. The biology of the honey bee. Cambridge, MA: Harvard Univ Pr).
small quantities of raw pollen directly, they and the queen depend on processed secretions (brood food and royal jelly) produced by nurse bees. Availability and quality of pollen can have a great influence on the health of the colony. Nectar is used as a source of carbohydrates and may be consumed directly or stored inside the hive and converted to honey.

Honey bees typically forage for food in the middle of the day within 1 to 2 miles (2 to 3 km) of the hive, but they may forage for 5 miles (7 km) or more if high-quality food is lacking nearby. From a risk assessment perspective, the large forage area of honey bees complicates the task of estimating potential exposure because the bees may come into contact with multiple pesticides. The time of day when foraging occurs in relation to pesticide application also complicates risk assessment and risk management. Numerous other factors should be considered in light of bee biology, which can impact the design or interpretation of data intended to inform pesticide risk assessment with these organisms.

2 Non-Apis Bees and Risk Assessment Implications

The biology and ecology of non-Apis bees differs from honey bees in several ways that may be important from the standpoint of risk assessment for pesticides. Most non-Apis bees are significantly smaller than honey bees, and if they are exposed to a spray application of plant protection products (PPPs, also known as “pesticides”) in the field, they receive a relatively higher dose because of their higher surface-area-to-volume ratio. Unlike honey bee larvae that feed primarily on secretions (brood food or royal jelly) from nurse bees, the eggs of most non-Apis species are laid directly on a “dough” of raw pollen and nectar, on which the larvae feed. That dough may contain much higher levels of pesticide contamination than the glandular secretions of nurse bees on which honey bee larvae feed. Many non-Apis bees forage earlier or later in the day or over a smaller area than A. mellifera, presenting different exposure scenarios. Foraging over a smaller area reduces opportunities for pesticides to be diluted with uncontaminated pollen or nectar before being delivered to larvae. In addition, the loss of a solitary non-Apis species in the field ends all reproductive capabilities for that individual, whereas the loss of honey bee workers in the field may have no impact on either survival or reproduction within the colony. Finally, non-Apis species that require nest-building materials, such as mud or leaf pieces, may be exposed if these materials are contaminated with pesticides.

There are more than 20000 species of non-Apis bees worldwide. Some non-Apis bee species are highly social, such as stingless bees, where several thousand individuals live in a perennial colony. Others, such as bumble bees (genus Bombus) are highly social, but typically have smaller, annual colonies of 40 to 400 or more workers. In temperate climates, bumble bee colonies are founded each spring by a new queen that has hibernated through a dormant winter season.
However, most non-*Apis* bees are nonsocial, solitary species (e.g., *Osmia lignaria*, the blue orchard bee) that go through an annual life cycle in which a single female bee conducts all the work of nest construction and nest provision with pollen and nectar. Most of these bees nest in earthen tunnels underground, whereas others nest in pre-existing tunnels in wood. Tunnel-nesting species need materials from outside the nest, such as mud or leaf pieces, to separate their brood cells, and these nesting materials could serve as a source of pesticide exposure.

As with honey bees, it is the female non-*Apis* bees that collect nectar and pollen from flowers as food for their offspring and, in doing so, generally transfer large quantities of pollen from flower to flower. Many horticultural crops, for example, blueberries (*Vaccinium*) or squashes (*Cucurbita* spp.), have evolved in the presence of fairly specialized non-*Apis* pollinators, and ongoing research across the globe indicates that native non-*Apis* bees play an important role in commercial crop pollination and a critical role in the pollination of native flora.

### Protection Goals for Decision Making

Risk assessment frameworks and the parameters used for risk assessment (studies, assumptions, process, etc.) shape the regulatory decisions, and therefore the conditions of use for a pesticide product. However, the broad goals articulated by the regulatory authority set the risk assessment framework and parameters and form the priorities that guide how a regulatory authority carries out its mission. Protection goals reflect scientific, legal, and other considerations important in defining priorities and objectives that the regulatory authority pursues on behalf of the communities they serve. Well-defined protection goals guide risk assessment by providing criteria for decisions within the risk assessment framework, from study design and interpretation to risk management and post-registration monitoring actions. Protection goals frequently are implicit in the mission of a regulatory authority and often are not explicitly restated at the start of a risk assessment. These goals, whether implicit or explicit, are carried out through the risk assessment process.

The desire to protect an environmental resource reflects several factors, such as

- the role and importance of a resource or organism in natural and cultivated ecosystems,
- the knowledge that the resource or organism is potentially exposed to plant protection products, and
- the ability to assess potential risk to a resource or organism using rigorous and transparent tools and methodologies in order to inform concerns and identify uncertainties.

In addition to reflecting scientific and legal considerations, protection goals must be clear, transparent, and proportionate to the level of concern for the
resource to be protected. Therefore, when a protection goal is articulated, we must have knowledge that the goal can be reached and supported through appropriate scientific analysis and actions (i.e., studies, assessments, management, and monitoring).

During the workshop, participants discussed the long-standing global importance of *Apis* and non-*Apis* bees in both commercial and noncommercial terms, and they proposed the following protection goals:

1. protection of pollination services provided by *Apis* and non-*Apis* species,
2. protection of honey production and other hive products, and
3. protection of pollinator biodiversity, that is, protection of adequate numbers and kinds of bee species that contribute to the health of the environment (primarily non-*Apis* bees).

From these protection goal statements, one can deduce

1. the value of bees (crop production, hive products, and biodiversity),
2. the spatial scale of the action (cropped and noncropped fieldscape), and
3. the biological entity to be protected (native and managed bees).

The protection goals listed above reflect the outcome of the reasoning and discussions during the workshop. Participants, however, acknowledge that it is the prerogative of respective regulatory authorities or organizations to define their own protection goals.

## 4 Overview of the Pesticide Regulatory Process

Globally, regulatory authorities have the responsibility to evaluate PPPs and the potential risks associated with their use. These authorities have risk assessment frameworks in place to assess the potential risk posed by these products to various species and taxa. However, with the introduction of new PPPs, changes in agricultural practices, and advances in the understanding of honey bee health and ecology, our ability to accurately characterize potential risks to insect pollinators, and in particular to the managed honey bee, has been seen as a challenge. While many countries share the same broad risk-based environmental assessment approach, differences among approaches account for national conditions, such as policies, legal requirements, or preferences.

The workshop participants considered a generic, tiered risk assessment methodology, and they worked to propose a process that included problem formulation, exposure and effects assessment, and risk characterization. A Tier 1 analysis is a conservative screen that efficiently separates those compounds that will not present a potential risk from those compounds that may present a potential risk. Higher-tier assessments refine the estimates or measures of potential exposure, potential effects, and the resulting characterization of risk. Assessors and
managers proceed through the risk assessment process (i.e., ascending through higher tiers of analysis) to determine whether the intended use of a compound is consistent with the protection goals of a regulatory authority. If the estimate of risk cannot be shown to be consistent with protection goals, then risk mitigation techniques may be implemented proactively to resolve concerns. (The workshop participants did not directly address risk management because it is technically outside the realm of assessment. However, it was briefly discussed as a component of the overall regulatory management of PPPs.) The components of a basic ecological risk assessment process are covered in detail in Section 10.

Current approach for assessing effects of pesticide products to pollinators

In the United States, the first tier of testing consists of an acute contact toxicity test⁴ on adult honey bees that provides a median lethal dose (LD₅₀), that is, the dose that causes death to 50% of the exposed organisms from a single dose of the test compound; any sublethal effects that may have occurred as a result of chemical exposure are also reported. In Canada, an acute oral toxicity test is also required when potential exposure exists. The acute LD₅₀ is assessed after 24 and 48 hours, but depending upon the outcome of the test, its duration can be extended up to a maximum of 96 hours, if necessary. Based upon the outcome of the acute LD₅₀ toxicity test, pesticides are classified as practically nontoxic, moderately toxic, or highly toxic to bees on an acute exposure basis. If the LD₅₀ is less than 11 µg/bee, additional testing may be required in the form of a foliar residue study to determine the duration over which field-weathered foliar residues remain toxic to honey bees. On a case-by-case basis, additional higher-tiered studies such as field pollinator studies with honey bees (i.e., hive studies) may be necessary if the data from toxicity studies indicate potential chronic effects or adverse effects on colonies.

In the European Union (EU), risk to honey bees from exposure to pesticides is determined on the basis of European and Mediterranean Plant Protection Organization (EPPO) guidelines, which include a 3-tiered progression of testing.⁵ EPPO guidelines describe laboratory tests (initial tier), semi-field (cage or tunnel) tests, and field tests for evaluating the lethal and sublethal effects of pesticides on adult honey bees. The testing approach in the EU is similar to that of the United States and Canada in that it consists of a tiered approach. In the United States, Tier 1 consists of an acute contact toxicity (LD₅₀) test on adult worker bees with the technical grade (relatively pure) active substance. In the EU and Canada, it is standard practice to conduct both acute oral and acute contact LD₅₀ studies on both the technical grade active substance and the for-

mulated end-use products (in cases where exposure to the end-use product itself is possible).

In addition to employing guideline toxicity test requirements, regulatory authorities around the world also make use of published open literature and dedicated studies of nontarget arthropods (NTAs) to evaluate the potential effects of pesticides on terrestrial invertebrates, or as a line of evidence to require higher-tiered testing. Along with guideline and open literature studies, regulatory authorities consider adverse effect reports (e.g., bee kill incidents) and monitoring studies in order to gauge the effects of pesticides on nontarget organisms.

**Trigger criterion and levels of concern**

A “trigger criterion” is a value, a threshold, used to define a limit of risk that is consistent with the protection goals of a regulatory authority. A trigger criterion or level of concern is compared to a quantitative risk estimate (e.g., hazard quotient [HQ] employed in Europe, or a risk quotient [RQ] employed in North America) to determine if the estimated risk is acceptable or not. If the comparison between a trigger criterion and an estimated risk indicates that the use of a compound is inconsistent with a protection goal, then it may be appropriate to either further refine the risk with additional data or seek action to mitigate potential risk. Trigger criteria and levels of concern are therefore policy tools; and as such, they are outside the realm of the SETAC Pellston Workshop and remain the right and responsibility of respective regulatory authorities to define.

In Europe, for example, when a spray formula is assessed, the trigger criterion at the screening level is where HQ ≥ 50. When HQ ≥ 50, either higher-tier data or risk mitigation may be sought. Review of pesticide bee-kill incidents in Europe shows that bee kills are rarely observed with compounds where the HQ < 50. Therefore, in Europe, this trigger value is used for sprayed products, as a screening tool to distinguish products of low concern from those for which a potential risk cannot be excluded. Workshop participants noted that while levels of concern promote efficiency in decision-making, risk assessment is an iterative process between risk assessors and risk managers and is comprised of multiple lines of evidence in order to determine whether the use of a compound on a specific crop is consistent with protection goals.

**Risk assessment for systemic compounds**

Many who are familiar with pesticide risk assessment recognize that the methodology and testing scheme employed for foliar application products (where exposure may be primarily through surface contact) is not adapted to assess potential hazard and risk from systemic pesticides. Bees are not expected to be subject to direct pesticide contact exposure during the use of many types of systemic treatments, such as those applied to the soil or as seed coats. However, the ability of these chemicals to be present in pollen and nectar during flower-
ing presents the potential for oral as well as contact exposure and therefore needs to be considered. The EPPO has recently put forward a risk assessment scheme for systemic compounds that includes the same tiered testing system, but replaces the HQ calculation with a toxicity exposure ratio (TER), where

\[ \text{TER} = \frac{\text{PNEC}}{\text{PEC}}. \]

The PNEC is the predicted no-effect concentration, while the PEC is the predicted exposure concentration. The PEC is determined from estimated or measured residue concentrations in the whole plant, flowers, pollen, or nectar. The dose that individual bees might ingest is then calculated for different categories of honey bees (e.g., larvae, queen, foragers), depending on the amount of contaminated pollen and nectar they may consume. PNECs are derived from acute, sublethal, and chronic toxicity data and may also include a factor to account for uncertainty. These factors range from 10 to 1, depending on whether the toxicity endpoint is assessed in a laboratory (Tier 1) or in a semi-field or field test; that is, uncertainty decreases as toxicity data become more representative of how the pesticide will be used.

5 Generic Problem Formulation for Pesticide Risk to Pollinators

Problem formulation is the foundation of an ecological risk assessment. Development of the problem formulation is an iterative process that generates the hypotheses concerning why ecological effects occur from human activities, articulates the purpose and objectives of the risk assessment, and defines the problem and regulatory action. Prior to the workshop, a subgroup of Steering Committee members prepared generic problem formulations for assessing risk to honey bees from 2 pesticide application scenarios: foliar spray application of a nonsystemic product and application of a systemic product to seeds or soil. While the problem formulation did not cover all scenarios of interest (e.g., non-\( Apis \) species), the exercise helped workshop participants understand the risk assessment process and the need to clearly identify, up front, the objectives and scope of the assessment. The Stressor Description box (p 13) includes the primary components that would comprise a problem formulation, for either a sprayed pesticide or a systemic soil or seed treatment.

6 Assessing Exposure of Honey Bees and non-\( Apis \) Bee Species to Pesticides

An essential component of an ecological risk assessment is predicting exposure to the target organisms that are being assessed. A subgroup of the workshop participants specifically explored the various pathways of exposure (both systemic and nonsystemic), the methods used to predict pesticide exposure, the
techniques employed to measure pesticide residues in matrices relevant for assessing bee exposure, and the use of higher-tier field study designs to refine bee exposure assessments.

Workshop participants agreed that the most significant routes of exposure to foliar-sprayed pesticides, for both honey bees and non-*Apis* bees, are through dermal contact and oral exposure of foraging adults, hive adults, and larvae to contaminated pollen, nectar, and processed food (e.g., bee bread, honey, brood

**Stressor Description**

Case 1: Sprayed product (Figure 1)
Case 2: Systemic product applied to the soil or as a seed coating (Figure 2)

**Management Goals:** The workshop participants identified the following as workable protection goals:

1) Maintain pollination services for agricultural crops and other valued plant communities.
2) Maintain ability of beekeepers to produce honey and other hive products.
3) Maintain pollinator biodiversity at the landscape level.

**Assessment endpoints** are explicit expressions of the actual environmental value that is to be protected. They should have ecological relevance, be susceptible to known or potential stressors, and be relevant to management goals and societal values.

For honey bees, relevant assessment endpoints are

- colony strength (population size and demographics) and
- colony survival (persistence).

Both of the proposed endpoints have ecological relevance, are known to be affected by pesticide use, and are directly relevant to the stated management goals.

**Measurement endpoints** are specific attributes of the entity (e.g., percent capped brood or hive weight), measured through a study and intended to be indicative of the assessment endpoints.

A conceptual model is typically a graphic model that identifies the potential routes of pesticide exposure to honey bees.

The risk hypothesis articulates how exposure may occur and effects may result. For honey bees, the risk hypothesis is as follows: Pesticide residues contact forager worker bees, which may in turn bring residues into the hive, which in turn can result in exposure to other workers, developing brood, or the queen, with the result that colony health or strength may be impacted if effects on individual bees are severe enough and last long enough.

The analysis plan identifies data needs and methods for assessing the risk hypothesis. This plan includes identifying the exposure and effects (toxicity) data needed and a method for combining these data to assess risk. Worst-case point estimates of exposure and effects (toxicity) are typically used in screening assessments. More definitive assessments may require consideration of the full range of possible exposure and toxicity levels, in order to predict the likelihood and severity of effects. Specific recommendations for measures of exposure and effects, and calculation of risk estimates, were developed during the workshop and are presented in Section 10. The efforts of the workshop participants were focused on elements of the analysis plan, including defining a risk assessment process, effects analysis, and exposure analysis.
Figure 1: Stressor source, potential routes of exposure, receptors, and attribute changes for a nonsystemic pesticide applied as a foliar spray (boxes with solid lines represent primary routes of exposure).

Figure 2: Stressor source, potential routes of exposure, receptors, and attribute changes for a systemic pesticide applied to the soil or as a seed dressing (boxes with dashed lines represent secondary routes of exposure).
food, and royal jelly). For systemic seed treatment and for soil-applied and tree trunk–injected systemic pesticides, the most significant route of exposure is through ingestion of residues in pollen, nectar, and processed bee food (e.g., brood food or royal jelly). Other potential routes of exposure include contaminated drinking water, hive material (e.g., contaminated comb wax), and inhalation. For non-Apis bee species, unique exposure sources may include contaminated soil (for solitary ground-nesting species and tunnel-nesting species that use mud to build cell partitions), contact with sprayed leaves, and contamination of nesting material made from leaves and petals. Workshop participants agreed that when the major routes of exposure are assessed, regulatory authorities should use methods that are conservative enough to protect other exposure routes. Unique potential exposure sources for systemic pesticides include dust from seed treatment, consumption of aphid honey dew, or possible consumption of guttation water.6

It is important that exposure routes which are formally assessed should be the same as those that were used to generate the toxicity endpoints available for use in an assessment. Therefore, exposure estimates for contact and dietary exposures are needed for both adults and larvae.

**Exposure estimates**

For contact exposure estimates of foliar-applied products, published residue data on insects can be used to estimate a predicted environmental dose (PED). For example, a PED could be used to refine a first-tier risk estimate built with an exposure value that is based on the application rate. The workshop participants agreed that further analysis would be necessary in order to select the appropriate data to be used to derive a PED calculation. In principle, a nomogram of contact exposure to honey bees and non-Apis bees could be developed using residue data from leaf-dwelling arthropods.7 When normalized for application rate, the resulting exposure value could be directly compared with acute contact toxicity data. Although data are available for flying insects, which would best represent exposure to honey bees in the field, participants in the workshop’s exposure subgroup recognized that relying only on flying-insect residue data could underestimate the potential exposure to non-Apis species. Therefore, data from leaf-dwelling species were considered as a source from which to develop a point estimate of contact exposure for foliar-applied products.

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6 Guttation water is derived from xylem sap and forms on tips or along the edges of leaves due to increased water pressure within the plant. Guttation may be a water source for bees. Pesticide residues have been measured in guttation water, indicating this as a potential source of exposure. (Girolami VM, Greatti M, Di Bernardo A, Tapparo A, Giorio C, Squartini A, Mazzon L, Mazaro M, Mori N. 2009. Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. J Econ Entomol. 102(5):1808–1815.)

7 For example, see data from Schabacker J, et al. 2005. Review on initial residue levels of pesticides in arthropods sampled in field studies. RIFCON GmbH Report No. RC05-029.
For predicting oral exposure to bees from foliar-applied products, there are limited data in the open literature on concentrations in pollen and nectar. However, workshop participants discussed the potential availability of data generated from semi-field studies conducted by pesticide registrants. The possibility of an industry coalition was discussed, where an effort could be formed that would aim at compiling pollen and nectar residue data from both open literature and proprietary sources to develop a separate nomogram used to predict concentrations in pollen and nectar on the basis of field application rates. Preferably, the nomograms would contain both mean and 90th percentile predictions.

Pollen and nectar residue levels, reported in terms of milligrams per kilogram (mg·kg⁻¹) can be compared to results from oral exposure toxicity studies if the results of the studies are based on concentrations in diet, that is, median lethal concentrations (LC50), or as a no-observed-effect concentration (NOEC). However, if the results from oral exposure toxicity studies are expressed as a median lethal dose (e.g., LD50 in micrograms per bee [µg/bee]), then the predicted exposure dose (in µg/bee) can be calculated on the basis of the concentrations in pollen and nectar and on reported consumption rates from different castes of honey bees.

For systemic compounds applied as seed coating, soil applications, or trunk injections, the most significant routes of exposure for adult and larval bees will be through ingestion of pollen, nectar, and processed pollen (i.e., brood food or royal jelly) and processed nectar (i.e., honey). Recognizing the limited field data available to develop exposure models, workshop participants considered the proposal by the International Commission for Plant-Bee Relationships (ICPBR) for a default value of 1 mg·kg⁻¹ in pollen and nectar as a potentially appropriate point estimate of exposure for a screening-level assessment (except for trunk-injection applications, which may produce higher residue levels). The proposed value was developed by ICPBR after residue data for systemic pesticides in plant tissue was compiled and analyzed. Once again, if the results from oral exposure toxicity studies are expressed as a dose (e.g., µg/bee), then the predicted dose can be calculated on the basis of the concentrations in pollen and nectar, coupled with reported consumption rates from different castes of honey bees.

**Higher-tier studies to refine exposure assessments**

When risk to bees is indicated on the basis of a screening-level (Tier 1) assessment, higher-tier studies with applications to bee-attractive plant materials are an option to refine exposure estimates. A contact toxicity study of residues on
foliage may be conducted. In such a laboratory study, a bee-attractive plant (e.g., alfalfa) would be sprayed with formulated product, and the bioavailability and persistence of toxic residues would be evaluated at various exposure time-points after application. The results could be used to determine the length of time between application and when bees could be safely exposed to residues on leaves or flowers of a treated crop.

Higher-tiered semi-field or tunnel tests are recommended to refine the oral exposure assessment, at the colony level, to both systemic and nonsystemic products sprayed on foliage. As discussed in Section 9, workshop participants believed that semi-field studies could use a bee-attractive crop such as *Phacelia tanacetifolia*, oilseed rape (*Brassica napus*), mustard (*Sinapis hirta*), or buckwheat (family Polygonaceae) to investigate exposure to a spray product. Use of these crop scenarios would provide a better opportunity to ensure exposure because the bees would have only the treated crop to forage on for a specified duration. Therefore, the results from a semi-field test would potentially provide data for a realistic, worst-case prediction of exposure of limited duration under actual field-use conditions. In these studies, pollen, nectar, bee bread (a mixture of pollen and honey), honey, and if desired, royal jelly and brood food can be collected and analyzed for residue levels. Unlike honey bee larvae that consume mostly processed pollen and nectar in the form of brood food or royal jelly, non-*Apis* bee larvae do consume pollen directly. Therefore, in studies using non-*Apis* bees, exposure measurements can be obtained directly via the stored pollen.

**Refining oral exposure of honey bees to soil-applied and seed-treatment systemic compounds**

A semi-field study is recommended for assessing exposure of honey bee colonies to systemic pesticides. In cases where the delivery of the systemic compound is through seed treatment, soil application, or trunk injection, the actual crop being assessed should be used (or potential worst-case exposure scenario when multiple crops are being considered) because there may be different rates of uptake, distribution, and metabolism of a compound in different plant species. Residue analysis should be timed to coincide with the highest nectar or pollen residues expected in the treated crop. Residues of systemic pesticides in leaves of trees may be highest several months after soil application, indicating that individual characteristics of the treated crop should be considered in assessing the residues in pollen and nectar. As in semi-field studies conducted with foliar spray products, residues in pollen, nectar, bee bread, honey, and if desired, royal jelly and brood food can be collected and analyzed for residues. The measured residue levels can be used in a refined risk assessment.
Refining exposure of non-Apis bees

If a screening-level risk assessment indicates potential risk, exposure as well as the effect of pesticides on non-Apis bee species can be refined using field or semi-field study designs. For assessing exposure to pesticides in pollen and nectar, solitary nesting bees such as blue orchard bees (O. lignaria) or alfalfa leafcutter bees (Megachile rotundata) can be used. However, nectar and pollen residue data gained from honey bee trials also can be used to assess exposure for non-Apis bees. Similar to studies with honey bees, for foliarly applied pesticides, studies with non-Apis bees should be conducted using a bee-attractive crop such as phacelia or sweet clover (Melilotus alba). Pollen and nectar can be collected directly from the foraging bees. Semi-field or field studies also can be conducted with Megachile to evaluate potential dermal or oral exposure through contaminated nesting material. For assessing exposure to systemic pesticides used as a seed treatment or applied as a soil treatment or trunk injection, a field study design can be used with the above non-Apis species to evaluate worst-case exposure because of the limited foraging range of these species. Potential exposure through soil also can be evaluated using these species. Many regulatory authorities have methods to estimate pesticide concentrations in soil, which may be used in such assessments.

In addition to understanding exposure pathways, such as concentration levels and duration of exposure, it is important to understand potential effects in terms of responses from different biological organization levels. Studies can be designed and conducted in a variety of settings, ranging from controlled (laboratory) to less controlled (semi-field or field), which represents a continuum of increasing realism.

7 Laboratory Assessment of Pesticide Hazards to Honey Bees and Non-Apis Bees

Laboratory assessment of pesticide hazards is the first step in a tiered-testing approach. Tier 1 testing is conducted on groups or individual bees and larvae under controlled conditions with well-defined criteria to yield statistically valid determinations of the intrinsic toxicity of active ingredients. Extrapolating effects observed on the individual to effects on the whole colony can be a challenge. Workshop participants identified test procedures that are well developed and offer the potential for wider harmonization, indicated new tests that are candidates for adoption, and provided perspectives on methods that require further development and research.

Adult honey bee acute-contact and oral toxicity tests are validated and therefore could be easily harmonized among many countries. Chronic toxicity tests for both adult and larval bees have seen recent improvements but require further development through ring testing before international harmonization could be
considered. (Recommended methods for these tests will be discussed in the full workshop report.) Extending similar tests to non-\textit{Apis} bees requires further protocol development and standardization, along with agreements on which species are most suitable for, or offer the most advantages for, laboratory, semi-field, and field testing. Sublethal impacts of pesticides on adults and larvae of both \textit{Apis} and non-\textit{Apis} bees are being documented in the scientific literature, but development of tiered species-specific tests requires significant effort and is seen as a high priority for future research.

**Harmonization of Tier 1 testing**

Currently, no globally harmonized, tiered testing system exists for honey bees. Participants in the workshop agreed upon the benefits of harmonizing the EU and US systems. The Organization for Economic Co-operation and Development (OECD) oral toxicity and contact toxicity tests for adult bees easily could be adopted and offer distinct advantages for improving risk assessment in the United States and other countries.

Elements of acute oral and contact tests for Tier 1 testing include

- the use of a toxic standard (e.g., dimethoate),
- the use of either multiple dose levels suitable for characterizing the dose–response relationship or a limit dose of 100 micrograms of test compound per bee,
- test duration to 96 hours, and
- observations of the number of dead bees and sublethal effects (e.g., abnormal behavior).

A significant historical database supports the successful use of these tests for foliar-applied products in the EU. Adult bee acute contact and oral testing provide the first measures of toxicity for the risk assessment of a sprayed product, whether it is a systemic or nonsystemic compound. The workshop participants agreed that acute and contact toxicity to adult bees should be measured with the active ingredient and in some cases, the specific product formulation, as a component of the Tier 1–level effects analysis.

**Tier 1 chronic adult and larval testing for honey bees**

For assessment of systemic products, the determination of a no-observed-adverse-effect concentration (NOAEC\textsuperscript{9}) for chronic exposure is an important improvement. Currently, although whole-colony field studies, which may assess

\textsuperscript{9} The terms no-observed-adverse-effect concentration (NOAEC) and no-observed-effect concentration (NOEC) are often interchanged, as is no-observed-adverse-effect level (NOAEL) and no-observed-effect-level (NOEL). Denotation of “concentration” verses “level” refers to whether the test substance was administered through the diet (concentration) or by gavage (level). All these terms refer to the treatment amount where no statistically significant response was noted in the treated organism relative to controls.
chronic exposure, are required by most regulatory authorities at higher tiers of refinement, no globally harmonized, or standardized, test protocol addresses chronic toxicity to adult bees or larvae. Research indicates that it is possible to conduct an adult bee chronic toxicity test for 10 to 14 days from which a NOAEC can be calculated. However, a standardized feeding protocol needs to be developed to ensure consistency and repeatability of this test. The workshop participants proposed that a chronic toxicity test with adult bees should be considered for inclusion as part of the Tier 1 test battery as soon as test methodologies can be verified. Measurement endpoints for a chronic adult test must be agreed upon, with measurements recorded at 24-hour intervals. With a specified protocol, the chronic test may produce a NOAEC or an effect concentration for a specified percentage of the organisms tested, that is, an ECx value. Possible measurement endpoints for a chronic study include the following: mortality, knock down (i.e., alive but immobile), staggering (i.e., moving but poorly coordinated), and responsiveness (i.e., hypo- or hyper-responsive).

The risk to bee brood has been investigated in the past only if the active ingredient was an insect growth regulator (IGR). There is currently no guideline or guidance document in the EU or the United States for a laboratory test that assesses chronic toxicity to larvae. However, a published test method has been partially validated in the EU through limited ring testing at bee institutes and contract labs. The workshop participants recommend that a Tier 1 chronic oral test with larvae be adopted as a standard Tier 1 test for all compounds where larval exposure is possible. Analogous to Tier 1 adult bee testing, consideration should be given to testing not only active ingredients but also specific product formulations in certain cases.

**Acute and chronic testing for non-**Apis** bees**

Protection of non-**Apis** pollinators is also a goal of the regulatory process for pesticides. There is uncertainty regarding the extent to which honey bees can serve as surrogates for the many non-**Apis** species. The development of standardized contact and oral toxicity tests for non-**Apis** species (adults and larvae) has yet to be completed and ring tested, but such development is seen as a highly desirable focus area for advancing the tiered testing system’s ecological relevance and for reducing uncertainty. Based on unpublished data on 21 different non-**Apis** species, it appears that LD50 values for several species are within an order of magnitude of the honey bee. Limited data for pesticides of newer chemistries suggest wider variations in the toxic levels (LD50) between **Apis** and non-**Apis** species. Further research and data are thus required to confirm whether the toxicity for adult non-**Apis** bees can be predicted from that for **Apis mellifera** adults.

Workshop participants agreed that the current contact toxicity-test protocol for honey bees can be readily adapted to bumble bees with some adjustments to the application method, that is, ventral dosing between the legs instead of on the hairy dorsum. Current oral dosing methods are less readily adapted from honey bees because individual solitary bees differ widely in their feeding and social interactions. Regarding other species, methods for laboratory rearing and toxicity testing of certain non-Apis species, for example, blue orchard bees, alfalfa leaf-cutter bees, bumble bees, and some stingless bees, are available or in development.

Sublethal effects on Apis and non-Apis bees

The sublethal impacts of pesticides on honey bee learning, behavior, and physiology have been well documented in the scientific literature. Yet, workshop participants agreed that further refinement in assessing and understanding sublethal effects on pollinators requires greater research in order to establish appropriate testing methods, to identify more uniform measurement endpoints (sublethal), and to determine linkages to existing regulatory authority assessment endpoints (e.g., impaired growth, reproduction, or survival). This is a challenging arena in which much progress has been made, yet much remains to be done.

Bioassays to investigate sublethal effects range from lab tests to orientation assays with free-flying bees in tunnels. Under laboratory conditions, the proboscis extension response (PER) has been used to measure the impacts of pesticides on associative learning. In maze tests, performance is measured by the bees’ ability to associate a visual mark with a reward of sugar water. With free-flying bees, performance is measured by their ability to make a path between release point and hive. Results from these types of tests have demonstrated adverse effects (i.e., lower performance) associated with sublethal doses of pesticides. To date, however, effects to associative learning have not been linked to colony-level performance. Additional work is needed in both laboratory and field test scenarios.

Semi-field and Field Studies with Honey Bees and non-Apis Bees

Semi-field and field studies can identify actual or potentially exposed organisms, routes of exposure, and adverse ecological effects, and such studies can provide evidence of a link between a certain stressor and an adverse effect. Therefore, these studies may be conducted if concerns have been raised in the initial tiers of risk assessment and further information is needed to allow for a more informed decision on potential risk to either Apis and/or non-Apis bees. Semi-field and field studies can be informed by lower-tier studies as well as other relevant sources of information; however, their precise design should be aimed at addressing specific issues raised in the initial tiers of risk assessment.
In developing guidance on semi-field and field studies for the honey bee, much use has been made of existing protocols in the EU. However, for non-Apis bees, there are no equivalents of the honey bee semi-field and full-field study protocols. Workshop participants, therefore, used their own practical and regulatory experience to provide further information on how such studies should be conducted with respect to both A. mellifera and non-Apis species. Workshop participants discussed elements of study design, for both semi-field and field studies, which include study objectives, the test organism, site selections and parameters, methods, endpoints, sample design, quality assurance and quality control standards, and the statistical analysis of the data. The participants thought it best to define the terms “semi-field study” and “field study.”

A semi-field study is performed on a crop that is grown outdoors in an enclosed test system with controlled or confined exposure. The crop is subject to good agricultural practices, and hence, there may be weeds present; but the predominant plant and thus the source of nectar or pollen must be the target crop. Nevertheless, the test system could be designed to reflect a desired exposure system and specific foraging environments, for example, a mixture of crop and weeds or flowering margins. The constituents of the test structure will depend upon the regulatory questions being asked.

A semi-field study is intended to evaluate effects from a worst-case exposure scenario, where bees are confined to plants treated with the target pesticide. Semi-field studies can be used to determine the following parameters:
- mortality,
- repellency effects or an impact on foraging activity,
- residual toxicity,
- effects on brood development, and
- colony strength.

A field study is performed on a crop that is grown outdoors with no enclosure. The crop is subject to good agricultural practices. The bees are free flying and able to seek out alternative food sources (a noted difficulty in the field-study test design). The test system should be designed to reflect the realistic foraging environment and exposure system in the field where the pesticide is to be applied. The constituents of the test structure will depend upon the questions being asked. A field study for a greenhouse situation should be conducted in a commercial greenhouse. Field studies can be used to determine the following parameters:
- mortality,
- residual toxicity,
- colony strength or over-wintering success,
• disease resistance,
• effects on brood development, and
• measurement of certain protection goals.

Outline of a semi-field study for *Apis*

Workshop participants felt that semi-field studies should be based largely on existing EU protocols (EPPO 170\textsuperscript{12} and OECD 75\textsuperscript{13}), paying particular attention to the size of test enclosures, crop, and colonies; the inclusion of appropriate test treatments; and the acclimation of the bees to the test enclosures prior to testing.

Key outputs (measurement endpoints) from a typical study may include
• mortality in the crop (through the use of sheets in the crop);
• mortality at the hive (through the use of dead-bee traps or sheets in front of the hives);
• foraging activity and other behavior;
• residues in pollen, nectar, pollen pellets, and dead bees;
• pollination deficit; and
• assessment of the brood, including an estimate of adults, the area containing cells, larvae and capped cells.

Interpretation of effects

If the protection goal is a pollination activity or function, then a semi-field study is capable of determining whether this is goal achieved through the use of foraging activity. If there is an adverse effect on foraging activity, then it may be necessary to determine whether the effects are realized at the field level. If the protection goal is honey production, then a semi-field study can be used to determine whether any observed effects (such as mortality, reduction in foraging, or other behavioral effects) are linked to honey production. Because this type of study is potentially a worst-case scenario in terms of exposure, it must then be determined whether any effects seen at the semi-field level are realized at the field level and, therefore, whether honey production actually could be impacted.

Guidance for non-*Apis* bees

As stated previously, there is no equivalent EPPO 170 or OECD 75 guideline for use in testing non-*Apis* bees in semi-field or field studies; however, information can be gleaned from some field studies conducted on NTAs in which pollinators are monitored. As a result, workshop participants proposed that if a regulatory question regarding a pesticide requires the assessment of effects on


non-*Apis* species, the study design should be developed on a case-by-case basis using EPPO 170 as a general guide. Study designs for blue orchard bees, alfalfa leaf-cutter bees, bumble bees, and some stingless bees are broadly in line with that for honey bees; however, differences exist and should be noted in specific study designs. Key outputs are mortality, foraging activity, and reproductive success. Care should be taken when evaluating and interpreting results from these studies until protocols are completely validated through ring-testing.

**Outline of a field study for (all Apidae) *Apis mellifera* and non-*Apis***

Field studies can be used to address a range of exposure scenarios and effects. Information and guidance on the general approach is available in the EU. The aim of a field study is to test the potential effects of a pesticide under realistic conditions in comparison to the laboratory environment or semi-field environment. The study should use hives or colonies with a minimum of 10,000 to 15,000 foraging bees. Colonies should consist of 10 to 12 frames and should include 5 to 6 brood frames.

Key outputs (measurement endpoints) from a field study could be the following:

- colony strength;
- weight of the hive;
- pollen, honey, and nectar stores;
- mortality at the hive (via the use of dead-bee traps or collecting sheets);
- mortality of drones and pupae;
- mortality in the crop;
- presence of the same queen;
- foraging activity in the crop;
- returning foraging bees (can be counted automatically);
- behavioral abnormalities;
- residues in pollen, nectar, pollen pellets, wax, bee bread, and dead bees;
- assessment of the brood, including an estimate of adults, the area containing cells, eggs, larvae and capped cells; and
- disease or pest levels (as a measure of resistance).

**Interpretation of effects**

Similar to semi-field studies, the interpretation of a measurement endpoint should be linked to the regulatory authority’s assessment endpoints and thus to protection goals. While field tests are considered the highest tier, and potentially the last point for prospective data generation, it is important that a final determination of the potential risk posed by a compound be based on the entire weight of evidence across all tiers of the assessment. If the protection goal is pollination activity, then a field study is capable of determining whether this is
achieved through use of data on foraging activity (which can include foraging for nectar and pollen), behavior, and mortality. Workshop participants pointed out that the measurement endpoints identified in the preceding sections are not direct measures of pollination activity; rather they are surrogate measures for the actual protection goal (pollination services or pollinator biodiversity). If on the other hand, the protection goal is honey production by the colony (primarily for *Apis* spp. and Meliponini), then a field study can provide information to assess this protection goal. For example, if there are clearly no effects in the field study, then it may be possible to infer that there will be no impact on honey production, provided other lines of evidence are consistent with this inference. If statistically significant effects are observed over the course of the study, then it can be concluded that the protection goal of no adverse effects on colony productivity (honey) may not be met.

**Role of monitoring and incident reporting**

Some countries have incident-recording schemes aimed at providing information on effects observed under actual use conditions. These incident schemes provide a measure of re-assurance (a feedback mechanism) that the regulatory process, including any associated trigger values, is appropriate. However, incident recording schemes are limited in that they are reactive, relying upon beekeepers, growers, or the public to take action. This reliance potentially leads to underreporting because beekeepers, chemical companies, or the public at large may be unwilling to report an incident for a number of reasons, including fear of reprisal. Cost-effective reporting schemes need to be developed which provide incentives to increase frequency and accuracy of incident reporting or noting effects from the field. Alternatively, prospective monitoring of colonies offers a means to obtain field exposure information that is useful for assessment and management. Both approaches are important for improving risk assessment and mitigation.

**Risk Assessment**

The risk assessment process is predicated first on defining protection goals, and then on identifying assessment endpoints that are indicative of the protection goals. Assessment endpoints are intended to be explicit expressions of the actual environmental value to be protected (such as the survival of a species). The selection of clearly defined assessment endpoints is important because they provide direction and boundaries in the risk assessment for addressing protection goals and risk management issues of concern. Measurement endpoints are specific attributes of the entity (e.g., percent capped brood or emergence weight) observed during a study and are intended to be indicative of the assessment endpoints. The workshop participants considered links between protection goals, assessment endpoints, and measurement endpoints (Table 1).
Analysis of potential exposure and effects provides the basic elements from which risk estimates are derived. Therefore, the ratio of exposure to effects remains the basis of quantitative risk characterization in the risk assessment process, where point estimates of both exposure and effects are used to develop point estimates of risk.

The proposed risk assessment process for pollinators is consistent with the basic tiered ecological risk assessment process (Figure 3) and consists of the phases identified in Section 5.0, that is, problem formulation, analysis (effects assessment and exposure assessment), and risk characterization. As the tiered process progresses, the components of risk (i.e., measurements of exposure or effects) are refined toward those that are more environmentally realistic. In addition to being tiered, the risk assessment process is also intended to be iterative. Risk assessors and risk managers can consider measures to reduce potential exposure and thereby reduce risk and the need to conduct higher-tier risk assessments. Screening-level assessments are intended to effectively and rapidly

- exclude substances of low risk concern from entering into resource-intensive higher-tier risk assessment and
- identify substances that may represent a risk to bees and for which a higher-tier risk assessment is needed.

In doing so, screening-level assessments enable regulators to more efficiently allocate resources and focus on those chemicals that are most in need of attention.
A first step in the risk assessment process is identifying potential exposure, without which a risk assessment is not needed. Therefore, the workshop participants identified exposure routes and defined them specifically for products applied by spray as well as for products applied by other methods, such as soil or seed treatment or injection. They provided a summary (Table 2) of the relative importance of different exposure routes of *Apis* and non-*Apis* bees.

Figure 3: Ecological risk assessment process
The main exposure routes identified for evaluation in the screening-level assessment are oral uptake of nectar and pollen and contact exposure. Not all exposure routes are included in the screening-level (Tier 1) risk assessment (e.g., wax and drinking water) because direct overspray and direct dietary intake via pollen and nectar are considered to be the worst-case (high-end) exposure. It will be necessary though to consider additional exposure routes, for example, residues in hive matrices, for higher-tier risk assessment purposes. Workshop participants believe that non-\textit{Apis} bee larvae may be exposed through contaminated pollen or through contact with residues in nesting material to a larger degree than the honey bee larvae (Table 2). Therefore, participants considered exposure to residues through nesting material (e.g., soil, plant materials) as a relevant source of exposure to pesticides for adult and larvae of non-\textit{Apis} bees.

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The workshop participants also agreed that measures of effect should include toxicity data (acute and chronic) for adult and larval life stages and should include effects data from \textit{Apis} and non-\textit{Apis} bees. Depending on the level of refinement needed in the assessment, the effects data may be at the individual organism level (Tier 1, or screening-level), based on laboratory toxicity studies, and in more refined assessments may include whole hives or managed non-\textit{Apis} bees, based on semi-field or field studies.

The proposed risk assessment process for insect pollinators exposed to residues on the surface of plants includes screening-level and higher-tier refinement levels (Figures 4 and 5, respectively).
The proposed risk assessment process for insect pollinators exposed to systemic soil and seed treatment–applied pesticides also includes screening-level and refined risk assessment processes (Figures 6 and 7, respectively).

Figure 4: Proposed insect pollinator screening-level risk assessment process for foliarly applied pesticides. Steps are numbered; arrows depict movement in response to a yes or no answer. A TER > trigger value = presumption of low risk; an HQ < trigger value = presumption of low risk. NTA = nontarget arthropod; see footnote 9, p 20 for NOEL.
In the deterministic risk assessment approach, the primary outcome of the risk characterization is the calculation of the risk estimate, for example, the RQ, the HQ, or the TER, depending on the country or region where the assessment

Figure 5: Proposed insect pollinator higher-tier (refined) risk assessment process for foliarly applied pesticides. Steps are numbered; arrows depict movement in response to a yes or no answer. A TER > trigger value = presumption of low risk; an HQ < trigger value = presumption of low risk.

In the deterministic risk assessment approach, the primary outcome of the risk characterization is the calculation of the risk estimate, for example, the RQ, the HQ, or the TER, depending on the country or region where the assessment
Figure 6: Proposed insect pollinator screening-level risk assessment process for soil- and seed-applied systemic pesticides. Steps are numbered; arrows depict movement in response to a yes or no answer. A TER > trigger value = presumption of low risk; an HQ < trigger value = presumption of low risk. NTA = nontarget arthropod; see footnote 9, p 20 for NOEL.

is being performed. The TER = hazard estimate / exposure estimate; both the HQ and the RQ = exposure estimate / hazard estimate. Therefore, while all are single-number or point estimates, assuming the same measurement units are
used for exposure and effects, they will be related to each other. A discussion
of any uncertainties, assumptions, strengths, or limitations associated with the
estimated risk, that is, the RQ or TER values, should be provided to character-

Figure 7: Proposed higher-tier (refined) risk assessment process for soil- and seed-applied
pesticides. Steps are numbered; arrows depict movement in response to a yes or no answer. A
TER > trigger value = presumption of low risk; an HQ < trigger value = presumption of low
risk.
ize the risk estimate. These will largely be discussed during characterization of the exposure and effects and will include refinement options used to ultimately determine the RQ or TER.

The risk estimate is interpreted through its comparison with a level of concern (LOC). An LOC, or a trigger value, is a number intended to demarcate a point, above or below which (depending upon whether an HQ or an RQ or TER is used), risks are considered to be potentially inconsistent with protection goals. Because a TER and an HQ are inversely related to each other, they will have opposite relationships with the trigger value. A TER that is above the trigger value indicates a presumption of low risk, whereas an HQ below the trigger value indicates a presumption of low risk. A risk estimate that exceeds an LOC or trigger value indicates potential risk and prompts the risk assessor or risk manager to consider the need for further testing or refinement or regulatory action, that is, risk mitigation. The LOC or trigger value is therefore a policy tool and is determined by respective authorities or between different jurisdictions.

As with risk assessments for other taxa, including humans, the assessment must consider available data using a weight-of-evidence approach and must clearly articulate the strengths and weaknesses (uncertainty and variability) associated with the risk estimates. An integral part of the assessment should be consideration of available incident data to determine whether effects observed under actual use conditions are consistent with those estimated through laboratory or field studies. If no risk is identified at the screening level, the process defined in this summary is considered to be sufficiently conservative to support a presumption of minimal risk to *Apis* and non-*Apis* bees such that additional refinement is not necessary. Conversely though, if after the recommended refinements in both exposure and effects assessments, the risk estimates still exceed regulatory LOCs, the process supports the presumption of risk to *Apis* or non-*Apis* bees that should be considered in risk management decisions.

The risk assessment process is intended to inform risk management decisions and is intended to be iterative (Figure 3). When sufficient data are available to reasonably predict that the intended use of a product is inconsistent with protection goals but the use of that product is considered efficacious and necessary, then a regulating authority may seek to manage the potential risk through mitigation. While not yet common to ecological risk analysis, distribution-based assessments of exposure and effects may be combined to estimate the magnitude and likelihood of an adverse effect, that is, probabilistic risk assessments. Probabilistic risk assessments provide the risk manager with a more thorough understanding of potential risks by creating more temporally and spatially explicit characterizations of risk. This information may better enable the risk manager to gauge the need for mitigation and to develop effective measures that reduce the risk of adverse effects. With respect to protecting *Apis* and non-*Apis* species, effective mitigation measures are especially critical for PPPs that are intended
to exert their pesticidal effects during the pollination period, because there is a
greater likelihood that bees will be exposed. Although deterministic risk esti-
mates typically are accompanied by well-defined LOCs, probabilistic risk assess-
ments typically do not have such well-defined combinations of likelihood and
magnitude of effect that may denote a risk of concern. In such cases, best pro-
fessional judgment on the part of both the risk assessor and the risk manager is
needed. Whether risks are estimated using deterministic or probabilistic meth-
ods, the presence of adverse effects in the field provides important information
about the risk of a compound, thus the importance of reliable, consistent, and
accurate incident reporting.

To the extent that a regulatory decision is made to register a pesticide product, that
decision should be consistent with the protection goals and objectives of
the authority, and mitigation language should be specified in a way that can be
implemented consistently both spatially and temporally. If mitigation language
fails to be clear enough for proper, consistent implementation and enforcement,
then inconsistent protection scenarios will result and the relationship between
the regulatory decision and the protection goals will be lost. In situations where
national-scale regulatory management methods (e.g., labeling requirements)
may be limited, it may be possible to manage potential risk at a regional, local,
or even at the landscape or field level through best management practices volun-
tarily employed by growers or applicators.

Analysis and Interpretation of Data Obtained from
Laboratory and Field Studies of Pollinators

During the course of the workshop, the participants identified a
need for additional statistical analyses of existing study designs and results to aid
in the design of future protocols and the conduct of future studies. This work is
being done outside the workshop under the direction of the steering committee.

An exploration of analytical methods most appropriate for evaluating pollena-
tor-related data, including study design, will serve both the regulatory authori-
ties, agrochemical registrants, and researchers engaged in such studies.

The workshop participants agreed on the need to create a common language,
based on mathematics, which can be used by all stakeholders when they discuss
the degree of effect on the individual bee, the colony, and the agricultural field
(e.g., through potential disruption of pollination services) that may result from
pesticide exposure. Their objectives are

1) to provide an overview of the advantages and limitations of quantitative
methods for assessing pesticide impacts to insect pollinators, including
model-based and hypothesis-based approaches, within a risk-based deci-
sion context; and
2) to provide information necessary to select appropriate analytical methods and gain insights into the interpretation of the statistical and model-based results.

Data from laboratory and field tests are to be used to illustrate and describe available quantitative methods that are appropriate for each level of biological organization. Such methods would include

- acute and subchronic larval honey bee toxicity tests,
- acute and chronic adult honey bee toxicity tests,
- semi-field tests at the level designed to assess toxicity at the hive level, and
- field tests designed to test temporal changes in hive dynamics and population growth.

For each test and biological level of organization, a rationale from a biological perspective is to be presented, including an explanation of the effects to pollinators the test is designed to represent. An explanation of the underlying mathematical model associated with the test data (laboratory and field) will be presented.

Experimental design issues including the number of tests, the number of test organisms, and the temporal and spatial issues in test construction are to be discussed and illustrated from a statistical perspective with case study data, as are power analysis, sample size, variance estimation, and temporal dependence.

The anticipated chapter will aim to provide clear and illustrative information linking the statistical results to the questions of interest within a risk-based decision context. The chapter will conclude with a description of how analysts can attempt to use the multilevel testing structure to link and interpret demonstrated individual effects with colony-level impacts within a risk-based decision paradigm. Finally, current study designs will be discussed, as will possible changes in the current framework, which may lead to improved decision-making.

11 Use of Ecological Models

Tests that focus on individual organisms deliver information on mortality or sublethal effects under laboratory conditions, but it remains unclear whether these effects impair the ability of an entire colony of honey bees to persist, to cope with other stressors, and to provide services such as honey production and pollination. Ecological factors such as adaptive behavior, population structure, exposure patterns, and landscape structure need to be taken into account. Additionally, for social insects like honey bees, the reproductive unit is not the individual worker bee, but the entire hive. The colony and its functioning can be considered as a complex net of buffer mechanisms that has evolved to increase the fitness of the queen and the colony. The loss
of individual worker bees might thus be less significant than in solitary-living species. On the other hand, buffer mechanisms have capacities and effects upon the capacity of compensatory mechanism stressors (e.g., Varroa mites, viruses, changes in landscape structure, or beekeeping practices) which are currently unclear. Semi-field and field studies allow for the inclusion and manipulation of some ecological factors, but certainly not all, nor do they allow for all the possible combinations of these factors. Field studies are expensive, are time-consuming, and can be inconclusive because of confounding factors that may be unavoidable in the field.

A model is a simplified representation of some real system, and ecological models provide a tool to overcome the limitation of empirical studies. Simplification is needed because the model should include only those processes that are most responsible for the internal organization of the system. In honey bee colonies, for example, the age at which in-hive workers change their task to foraging depends on the success of the actual foragers. This feedback mechanism is likely to affect the ability of a colony to cope with variability or stress in forage availability, and it therefore should be included in the model. In the context of regulatory risk assessment, ecological models are often grouped with individual-level models addressing toxicokinetics and toxicodynamics (TK-TD) or dynamic energy budgets (DEB) to mechanistic effect models.

Existing models of honey bee colonies focus on the interaction among parasites, for example, Varroa mites, climate, forage, pesticides, and in-hive temperature gradients. None of the existing honey bee models seems suitable for regulatory risk assessment because

1) none of these models is linked to an explicit representation of foragers and the landscape, and none of them considers multiple stressors simultaneously;

2) some of the models are not fully documented; and

3) most of the models have been insufficiently tested (e.g., there are no sensitivity analyses, too few simulation experiments that help us understand the controlling factors in the model, and too few comparisons to patterns observed in reality).

Ecological models of non-Apis pollinators have been developed for a few species, but most of them have been developed for purposes other than pesticide risk assessment.

Ecological models for honey bees and other pollinators hold great potential to answer questions that cannot be answered with individual-level tests and semi-field and field studies. Promising models are under development, but further modeling projects and appropriate funding opportunities would be more than worthwhile because of the extreme importance of and interest in the health and protection of pollinators.
Risk Mitigation and Performance Criteria for Risk Management

A regulatory authority may seek to address potential risks through the implementation of risk mitigation measures. Much risk management, that is, risk mitigation, is accomplished through measures taken to reduce or avoid exposure. The regulatory authority may mitigate the potential risk by denying registration on a particular crop or use site, or by modifying the manner in which a product is used. Mitigation language, captured on labels, should allow for consistent interpretation and implementation. Improper and inconsistent implementation is likely to lead to inadequate protection and misuse. If a potential label violation involves detailed investigation by a third party, the clarity of the intended use and restrictions associated with a product label is necessary in order to establish misuse.

It is important to understand the specific characteristics of the risks that need to be mitigated when risk management is pursued. Specific characteristics of potential risk may include whether the concern is related to

- effects that are acute or chronic or both, on adult bees or immature life stages or both, or on individual bees or entire bee colonies or both;
- honey bees, other species of bees, or both;
- the crop or site being treated, to off-target movement onto adjacent crops or blooming weeds, or to other concerns (such as contamination of nesting materials used by non-*Apis* bees);
- foliar application, soil application, or both;
- whether the pesticide has an extended residual hazard to bees (more than 8 hours); or
- whether the exposure may be limited or avoided.

Central to managing pesticide risk to bees is controlling potential exposure at the time, or under conditions when bees are, or are likely to be, present at an agricultural site. These conditions may include the presence of bees on the commercial crop, on the understory material, or on adjacent forage material. Every attempt should be made to avoid applications of insecticides and fungicides during bloom or during the bee foraging periods. However, use of a PPP may be specifically needed (or designed for use) when one of the aforementioned conditions exists. In these cases, attention must be given to all options to reduce risk to the pollinator and to the crop. Variables such as timing of application to the target crop, management of the understory and the border area, as well as elements of agricultural practice could be examined as avenues to manage exposure and risk.

In the case of honey bees, communication and cooperation among growers, applicators, and beekeepers is perhaps the most important tool to reduce risk and to ensure that the needs of all parties are met. Growers and beekeepers engage
in reciprocal endeavors; it is therefore to the advantage of each to anticipate the concerns of the other. Cooperation and understanding of one another’s needs is essential.

13 Research and Recommendations

Throughout the workshop discussions, participants identified key areas where additional research may lead to improvements in the pesticide risk assessment process for pollinators. Below is a brief summary of recommendations for further research or collaboration.

Exposure nomogram for pesticide concentrations in pollen and nectar
Workshop participants discussed the possibility and value of an industry coalition to compile pollen and nectar residue data, from both published and proprietary studies, in order to develop a nomogram that can be used to predict concentrations in pollen and nectar on the basis of application rates. Such a nomogram would present parameters necessary to describe the distribution of concentration values, including high-end values (e.g., 90th to 95th percentiles) that may be suitable for use lower-tier risk assessments.

Exposure data from trunk injection
Further data are needed to appropriately describe the range of expected residue concentrations in nectar and pollen following the trunk-injection application method. A database built to explore residue range, varying application protocol, test substance, and tree species potentially could be a very important tool for stakeholders.

Likelihood and magnitude of pesticide exposure through guttation
Workshop participants recognized the uncertainty around guttation as a source of systemic pesticide exposure. The workshop participants recommended that research be conducted that would allow a more informed analysis of whether this route of exposure should be considered in pesticide risk assessment for pollinators.

Pesticide fate within the colony
Information on the movement, distribution, and repartitioning of pesticides within a honey bee colony would be important for predicting exposures to different castes of bees. This information also would be useful in determining how many samples should be analyzed to obtain a robust and repeatable measurement of residue levels in various matrices. It would also be helpful in determining how many colonies should be sampled within an apiary to get accurate representation of the apiary, as well as a comparison of residue levels in pollen pellets to beebread.
Modification and validation of larval test
Workshop participants noted the need for adoption of a larval (early life stage) test. A published method\(^{14}\) has gone through some validation in the EU, with limited ring testing. The participants discussed potential modifications to this test to improve throughput and reduce costs. Further development and validation of this test method would be useful for regulatory authorities.

Standardized protocol for chronic feeding study
Workshop participants noted the need for a standardized protocol for a laboratory-based chronic feeding study with adult bees.

Testing method to assess effects on foraging behavior
The need for further research on methods (test design, measurement endpoints, statistical considerations, etc.) to evaluate potential pesticide effects on honey bee foraging behavior was identified. Workshop participants noted several current experimental designs; however, a standardized test would be an important component of a pesticide risk assessment framework for pollinators.

Artificial diet for larval testing
Workshop participants noted that the use of royal jelly for feeding larvae represents a major difficulty in larval testing. Research into an artificial diet for rearing honey bee larvae would greatly contribute to the larval testing approach.

Toxicity testing for non-\textit{Apis} species
Research into the necessary adaptations of honey bee toxicity-testing methods for application to non-\textit{Apis} species, and validation of those methods through ring testing, are needed before these species can be successfully integrated into and used in tiered testing for risk assessment.

Improvements to monitoring efforts
Cost-effective reporting schemes that provide incentives to all parties involved, that is, beekeepers, applicators, and growers, to help increase accurate reporting of experiences from the field would be an important improvement to the pesticide regulatory framework (i.e., risk assessment and risk management). Further, a common platform for incident reporting among regulatory authorities would facilitate the sharing of incident data and management strategies.

Research on effects of pesticides on community or landscape populations
A need exists to better understand the landscape- or community-scale impact of pesticide use. Both empirical-based research and community modeling would be valuable approaches that could potentially inform management strategies.

Modeling development and refinement
Further research and work on model development for use in pesticide risk assessment for pollinators is needed to document and refine a model’s biological realism, sensitivity, robustness, parameterization, and calibration. Models could be used to explore links between measurement endpoints and assessment endpoints, or across protection goals. Collaboration among modelers and others such as regulators or entomologists would help direct model development and refinement.
Pesticide Risk Assessment for Pollinators:  A SETAC Pellston Workshop

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• Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis.
• Support the development of ecologically acceptable practices and principles.
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• Publish scientific journals, a newsletter, and special technical publications.
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