10 Overview of a Proposed Ecological Risk Assessment Process for Honey bees (*Apis mellifera*) and Non-*Apis* Bees

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10.1 INTRODUCTION

Ecological risk assessments are intended to evaluate the likelihood that adverse ecological effects may occur as a result of exposure to one or more stressors (USEPA, 1992). Typically, at the first tiers, risks are evaluated for individual taxonomic groups (e.g., freshwater fish, upland game birds, or terrestrial plants) using surrogate species. At higher levels of refinement, risks to individual taxa may be further integrated to determine whether there are effects to the community. However, risk assessments are typically conducted at the taxon level (USEPA, 2004). The intent of this chapter is to describe a proposed method for estimating risk to honey bees (*Apis mellifera*) and non-*Apis* bees from pesticides that are applied via sprays (acting on contact) and via seed/soil treatments and tree trunk injections (acting systemically).

In general, a pesticide risk assessment process is used for evaluating new compounds or new products entering the market or those compounds undergoing re-evaluation, as in the 10-year process of re-evaluation in the European Union (EU) or in North America where chemicals are re-evaluated every 15 years. As with risk assessments for other taxonomic groups, the proposed risk assessment method described in this document makes use of surrogate species. The ecological risk assessment process described consists of a series of steps or phases, which are intended to be iterative where information gathered at each step is evaluated against the protection goals. The risk assessment process consists of a *problem formulation* (Phase 1), *analysis* (Phase 2) and *risk characterization* (Phase 3). This generic process is depicted in Figure 10.1. In Phase 1, *problem formulation*, measurement endpoints are identified in relation to protection goals and corresponding assessment endpoints, a conceptual model is prepared and an analysis plan is developed. Based on the conceptual model and its associated risk hypothesis, the analysis plan articulates how the risk hypothesis will be tested. In Phase 2, *analysis*, available measures of exposure and measures of effect are evaluated. Through environmental fate data, the movement of a stressor (i.e., the pesticide and relevant transformation and breakdown products) in the environment is characterized; this is frequently termed the exposure characterization or exposure profile. Similarly, the potential acute and chronic effects of a chemical are characterized in what is frequently termed the stressor-response profile. Additionally, the proposed and/or existing uses of a compound are characterized and, based on these uses and the environmental fate of the compound, predicted/estimated environmental concentrations (PEC or EEC) are derived.

Once effects and exposure are characterized, the risk assessment proceeds to Phase 3, *risk characterization*. Typically, the risk characterization consists of two steps, that is, risk estimation and risk discussion (evaluation). In the risk estimation step, the measures of exposure (e.g., EECs or PECs) and measures of effect are integrated to develop risk estimates. These risk estimates may be based on point estimates of exposure and a point estimate of effect, for example, for Tier 1, exposure is based on application parameters assumed to result in the highest exposure for a particular use, and point estimates of effect, for example, the acute median lethal dose to 50% of the species tested (LD50). If initial values for potential exposure and effects result in risk estimates that exceed regulatory triggers, then these point estimates can be refined through higher tier testing with regard to both potential exposure and/or potential effects. Possible refinements in exposure estimates are discussed in Chapter 7 while possible refinements in effects are discussed in Chapter 8 (laboratory studies) and Chapter 9 (semi-field/full field studies). As ecological risk assessment methodologies evolve, refined estimates could be based on distribution-based estimates of either exposure (e.g. residue concentrations in pollen from field monitoring studies based on application rate reflecting the worst case for a particular use), or effects (e.g., species sensitivity distribution using LD50 values for non-*Apis* species).

Regardless of whether point estimates or distribution-based estimates are used, the integration of exposure and effects data is typically expressed as a ratio (quotient), and it is this ratio that is considered to be the "risk estimate." If point estimates of exposure and effects are used as inputs, the risk quotient (RQ) is a deterministic point estimate of risk. If the exposure and/or effects inputs are probability distributions of possible values, the risk estimate is itself a "joint" probability distribution and represents a probabilistic estimate. Deterministic

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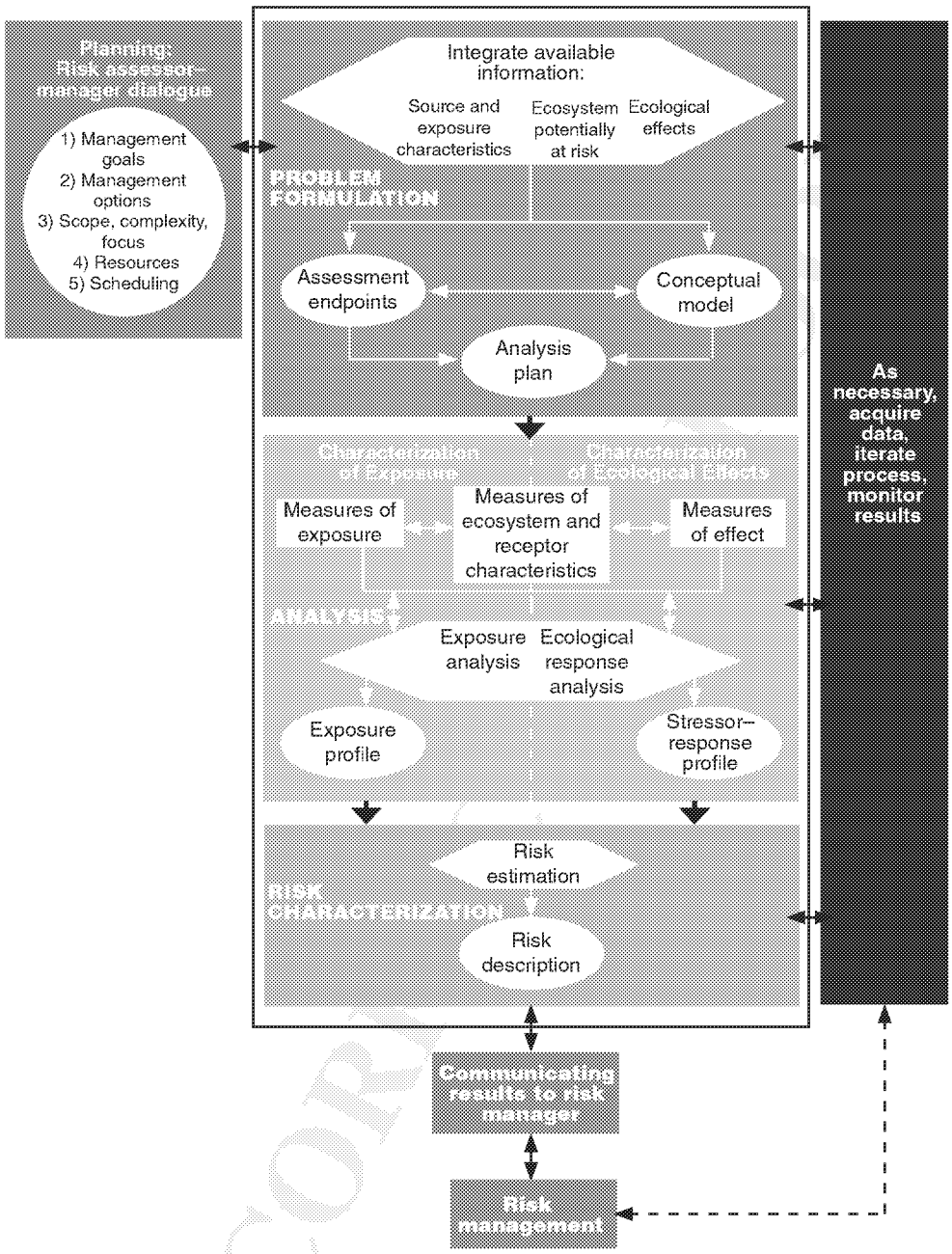

FIGURE 10.1 Diagram of ecological risk assessment process employed by USEPA.

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estimates of risk, based on point estimates of exposure and effects, do not typically provide information on the magnitude and likelihood of adverse effects. This uncertainty is in most cases accounted for with the use of assessment factors. In refining the risk assessment on the basis of distribution-based estimates of either or both exposure and effects, probability distributions and particularly joint-probability distributions allow the estimation of both the likelihood (probability) and magnitude of an adverse effect (e.g., estimates of a 40% chance that 60% of the species will be affected). The decision to move from point-estimate-based approaches to distribution-based approaches[1] that may also be spatially and temporally specific is predicated on the risk manager's need for additional information to support their decision and the availability of data to support such approaches.

The second part of *risk characterization* is risk evaluation, where quantitative estimates of risks are, when necessary, further described using qualitative data. Multiple lines of evidence are used to more fully describe what is known about potential adverse effects resulting from the use of a pesticide. Risk evaluations include additional discussion about the variability associated with the measured endpoints along with associated uncertainties, that is, attempts to characterize what is not known. When necessary or possible, the intended effects of relevant mitigation measures may also be discussed. Any incident data, that is, adverse effects reported involving the actual use of the compound in the field, are also discussed to further characterize potential effects.

Although the risk assessment process is depicted as three distinct phases, each phase is intended to be iterative. As more information (data) becomes available, the outcome of the process should evolve to accommodate the data. 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 be refined as more information becomes available. A critical component to this iterative process is clear communication between the risk assessor and the risk manager to ensure that protection goals are adequately articulated and that the relevant mitigation measures on risk estimates may be implemented and potentially evaluated within the risk assessment.

Consistent with the iterative nature of the risk assessment process, regulatory authorities typically rely on a tiered process for conducting ecological risk assessments; the preliminary, or screening-level (Tier 1) assessments are intended to screen substances for which a potential risk cannot be excluded. Higher tiers attempt to refine risk estimates to (1) identify whether a potential risk will likely be encountered under more realistic assessment conditions, that is, using less conservative assumptions regarding potential exposure and effects; (2) determine the conditions under which potential risks may occur; and, (3) identify the spatial and temporal characteristics of risks. The tiered risk assessment process identifies those chemicals for which a higher level of resources should be devoted to support more refined and detailed assessments. It should be noted though, that while probabilistic tools can be used to refine estimates of exposure and effects, and to quantify spatial and temporal characteristics of risks, they are not typically applicable to determining the conditions of occurrence for risk. Additionally, such refinements are typically focused on specific uses which have exceeded trigger values and which require a more detailed understanding of the potential magnitude, likelihood, and/or duration of a particular effect.

Decision criteria are used within a tiered framework as a basis for discriminating potential risks among substances. Screening-level risk assessments may have predetermined decision criteria to answer whether potential risks exist, as for example in the EU where decision-making criteria are defined for all groups of organisms (EC, 2001). Conversely, higher tier risk assessments may not have predetermined and/or uniformly defined decision criteria since the management decision may change from yes/no to questions

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[1] Species sensitivity distributions are an option to refine the evaluation of effects for risk assessment performed for a group of organisms and not at the level of a species, for example, the honey bee.

regarding "what, where, and how great is the risk," as for example in the United States (USEPA, 1998) and may also be associated with restrictions/conditions intended to limit the risk (which is the case in both the EU and United States).

In the following sections, the risk assessment process for honey bees and non-*Apis* bees is described. Consistent with the tiered process discussed in the preceding sections, the following sections propose risk assessment flowcharts discussed during the Workshop and are intended to illustrate the different steps mentioned above. Each step of these risk assessment processes are then discussed in greater detail, starting with screening-level assessments (Tier 1) and proposed refinements that incorporate additional data on potential exposure and effects to both *Apis* and non-*Apis* bees. The proposed process is delineated for pesticides that are applied foliarly and act on contact with or ingestion by insects. A different risk assessment process is articulated for pesticides that are applied to soil or as a seed treatment. For soil and seed treatments that are systemic, the chemical is taken up by the plant and distributed either through the xylem (i.e., translocation through the plant in the direction of xylem flow (acropetal[2]) or through the plant phloem (i.e., translocation through the plant in the direction of phloem stream (basipetal[3] and acropetal). The route of exposure to systemic compounds applied as soil, seed, or tree trunk injections is primarily through ingestion of residues in pollen and/or nectar.

10.2 PROTECTION GOALS, ASSESSMENT AND MEASUREMENT ENDPOINTS, TRIGGER VALUES FOR TRANSITIONING TO HIGHER LEVELS OF REFINEMENT, AND RISK ASSESSMENT TERMINOLOGY

As previously discussed, the initial phase of a risk assessment process is problem formulation. Problem formulation articulates the intent of the risk assessment and is predicated on particular protection goals for which the regulatory authority is responsible. In order to build a proposed risk assessment process for pollinators, the participants of the Workshop identified plausible, surrogate protection goals, that included:

i. protection of pollination services provided by *Apis* and non-*Apis* species'
ii. protection of honey production and other hive products; and
iii. protection of pollinator biodiversity,

In order to structure an assessment that allows addressing risk management concerns, that is, realize protection goals, it is important to define assessment endpoints. Assessment endpoints are intended to be explicit expressions of the actual environmental value that is to be protected and are operationally defined by an ecological entity and its attributes (USEPA, 1998). For assessing potential risks to *Apis* and non-*Apis* bees the ecological entities would be the organisms themselves (e.g., larval and adult honey bees and bumble bees) and potential attributes would consist of survival, development, and reproduction. The ability of assessment endpoints to support risk management decisions depends on the extent to which they target susceptible ecological entities and measurable ecosystem characteristics (USEPA, 1998). Protection of the growth, reproduction, and survival at the colony/population level of these species will conserve pollination services, biodiversity contributed by pollinators, and availability of hive products (e.g., honey production). The conventional assessment endpoints of survival, development, and reproduction can be articulated for

[2] Acropetal refers to the direction of movement and is typically intended to denote movement from the base of a plant (e.g., roots) toward its apex.

[3] Basipetal refers to the direction of movement and is typically intended to denote movement from the apex of a plant toward its base.

Apis and non-*Apis* bees to include colony size and survival for honey bees, and population size and survival for non-*Apis* bees.

Assessment endpoints are further defined by measurement endpoints. Measurement endpoints are attributes that are examined at the study level which, taken either individually or together, are indicative of an assessment endpoint. In initial (screening level) laboratory studies, it is practical to measure endpoints such as individual survival, toxicity to and developmental effects on larvae (brood), and behavioral effects (e.g., effects that become manifest in adults due to exposure as larvae). These measurement endpoints are relevant because if severely impacted, they can result in effects at the colony/population level and can be indicative of the ability of a colony to grow, reproduce, or survive. In higher tier tests, it may be possible to directly measure effects on colony/population size and viability. However, as noted in previous chapters, further research is required to ascertain which, and at what level (sublethal) effects is indicative of a colony-level, or population-level effect. The linkage between protection goals, assessment endpoints, and possible measurement endpoints are presented in Table 10.1.

The terminology of risk assessment can be confusing due to the differences amongst regulatory authorities. Many parts of the processes outlined in this document make reference to the European and Mediterranean Plant Protection Organization (EPPO) methodology, and the testing methods for non-target terrestrial arthropods thereof. Table 10.2 presents the different risk expressions used herein.

Note that in Tier 3 analysis, where a field study is performed, neither a hazard quotient (HQ) or RQ, nor a toxicity exposure ratio (TER) is calculated. Rather, effects are characterized, statistically significant or not, in the context of actual exposure conditions and in the context of whole hive biology.

TABLE 10.1

Linkage of Protection Goals, Assessment Endpoints, and Measurement Endpoints for Social Bees (Including *Apis*) and Solitary (Non-*Apis*) Bees. Initials (L) and (F) Designate Endpoints Most Applicable to Laboratory (L) Studies and Field (F)

Protection Goal	Assessment Endpoints	Measurement Endpoints Population Level or Higher	Measurement Endpoints Individual Level
Pollination services	Population size and stability within the crop and/or its boundaries	Social bees: colony survival (F), colony strength (F) Solitary bees: population size (F) and persistence (F) over time	Social bees: individual survival (L, F), fecundity (F), brood success (L, F), behavior (L, F) Solitary bees: individual survival (L, F), reproduction (F), behavior (L, F)
Hive products (honey, etc.)	Production of hive products	Production of hive products (F)	Individual survival (L, F), brood success (L, F), behavior (L, F)
Pollinator biodiversity	Species richness and abundance on the crop/in the boundaries	Colony survival (F), colony strength (F), brood success (F), behavior (F) Species richness and abundance (F)	Individual survival (L, F), brood success (L, F), behavior (L, F)

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TABLE 10.2
Risk Estimates and Their Components Used by Regulatory Authorities

Ecological Risk Estimate	Effects Component	Exposure Component	Comment	Where/How it is Used
Hazard quotient (HQ): effects/exposure	LD50 measured as μg/bee	Dermal exposure concentration or oral dosing concentration as g/ha	Numerator and denominator are expressed in dissimilar measurement units	Used in European assessments Used in Tier 1 analysis
Risk quotient (RQ): exposure/effects	LD50 measured as μg/bee	Contact exposure concentration, or oral dose concentration	Numerator and denominator are expressed in same measurement units	Used in North American assessments Used in Tier 1 analysis
	No observed adverse effect level (NOAEL) measured as μg/bee	Oral feeding concentration (solution) or dietary intake (pollen or nectar)	Numerator and denominator are expressed in same measurement units	Used in North American assessments Can be used in Tier 1 and Tier 2, analyses
Toxicity exposure ratio (TER): exposure/effects	LD50 or the no observed adverse effect level (NOAEL) measured as μg/bee	Oral feeding concentration (solution) or dietary intake (pollen or nectar)	Numerator and denominator are expressed in same measurement units	Used in Tier 1 analysis (for larvae) and Tier 2 analysis

10.3 RISK ASSESSMENT FLOWCHARTS

This section illustrates the proposed risk assessment process identified by the participants of the 2011 SETAC Workshop on Pesticide Risk Assessment for Pollinators. The decision process is described and depicted in flowcharts to better highlight the progression of events through the tiers. Risk assessment starts with a preliminary verification that a risk assessment is warranted by first describing the routes of exposure that are considered likely and will trigger further evaluation. This leads to screening steps intended to exclude situations where the potential for adverse effects is considered low and with a sufficient margin of safety to conclude no further analysis is necessary. The process then focuses on uses for which further characterization of the risks is necessary and guides the assessor in efforts to identify the necessary data to enable the estimation of effects and exposure levels needed to assess potential risks from these scenarios.

An overview of each step in the problem formulation and risk assessment process, that is, screening-level assessment to more refined evaluation of effects and exposure based on laboratory data, to higher tiered assessments involving semi-field and field studies can be found in Chapters 5 and 6. Efforts to refine risk estimates are typically predicated on refining estimates of potential exposure and effects. For detailed descriptions of the studies to be undertaken to generate these data, refer to Chapter 7 (assessing exposure), Chapter 8 (laboratory-based effect studies), and Chapter 9 (field-based effect studies).

The flowcharts below are used to depict a generic risk assessment process that was developed during the Workshop. Two proposed processes distinguish between compounds applied as spray for which the worst case exposure may be expected through direct contact of pollinators with spray droplets during the flowering period (Figures 10.2 and 10.3) and, products used as soil or seed treatments for which an exposure may occur as a result of the systemic properties of the compound or its degradates (Figures 10.4 and 10.5). It is important

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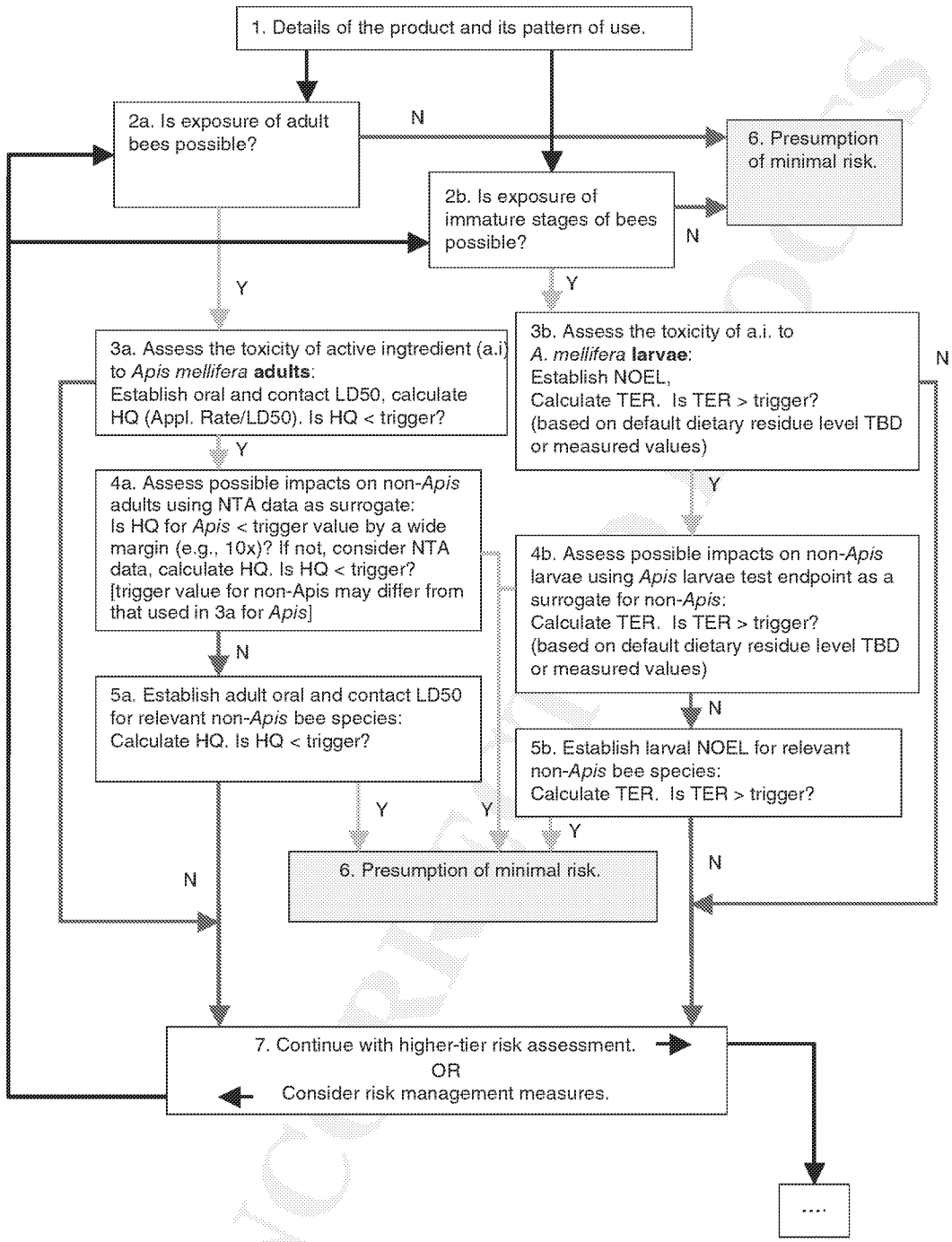

FIGURE 10.2 Insect pollinator screening-level risk assessment process for foliarly applied pesticides. (For a color version, see the color plate section.)

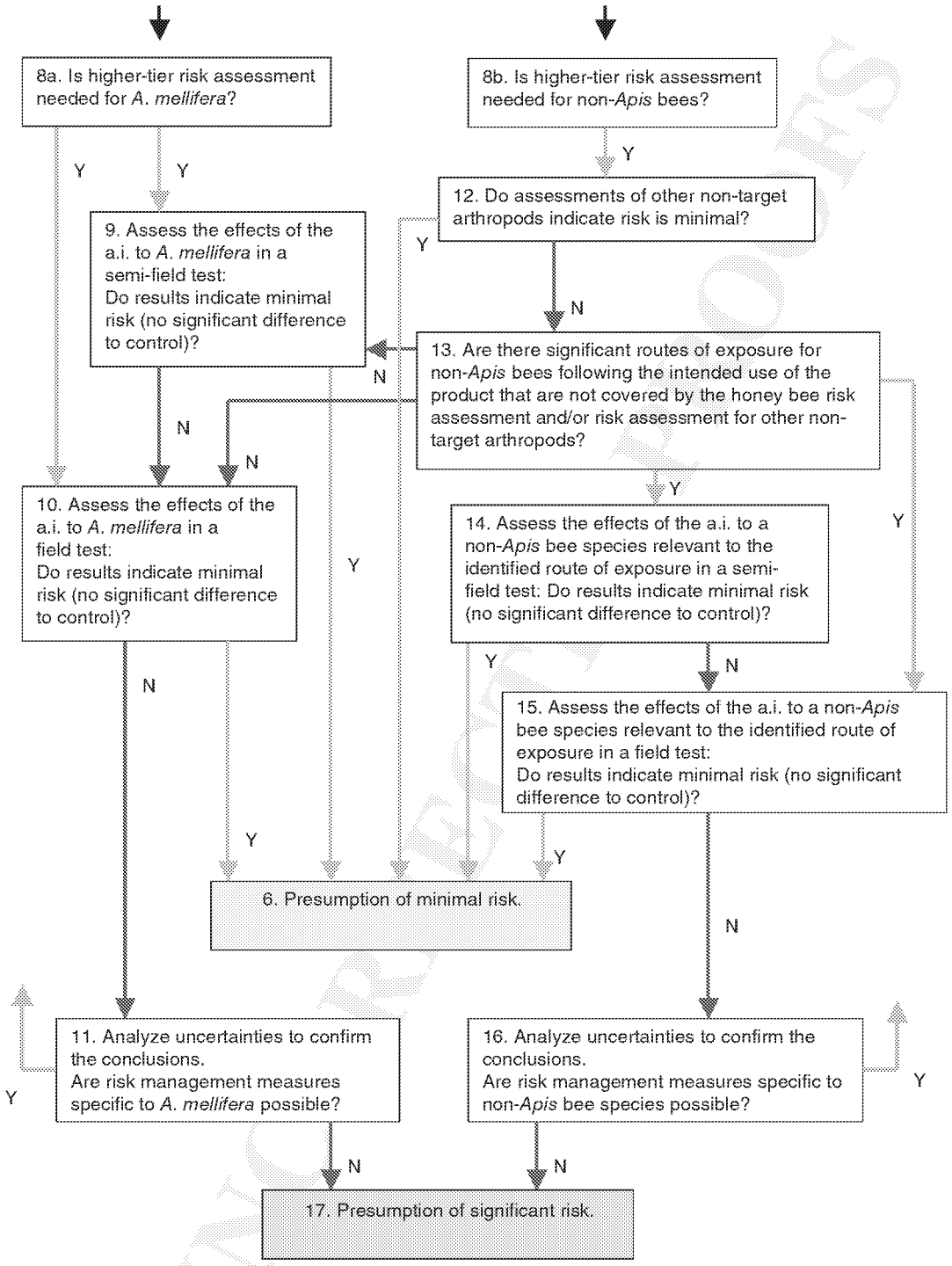

FIGURE 10.3 Higher tier (refined) risk assessment process for foliarly applied pesticides. (For a color version, see the color plate section.)

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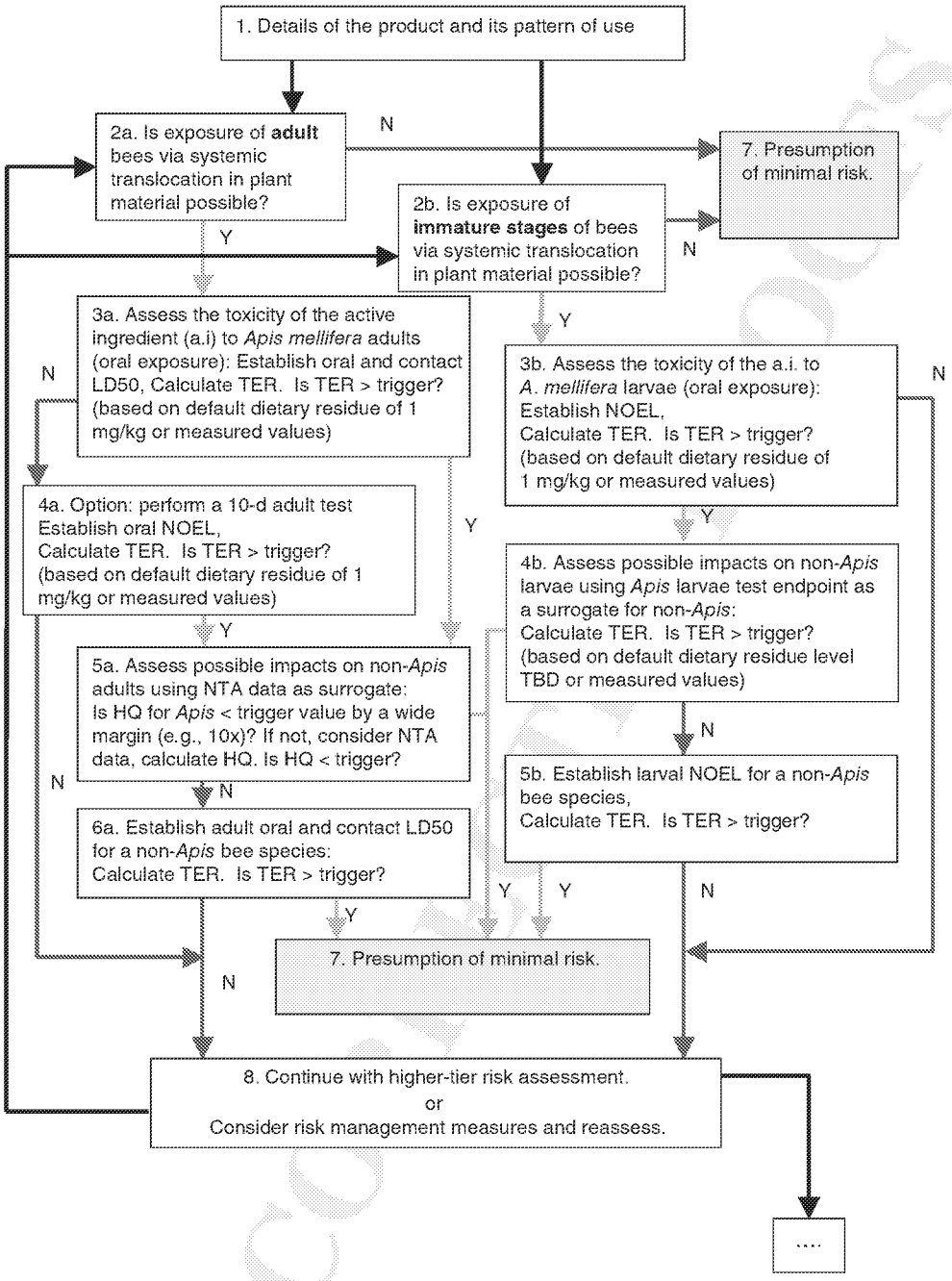

FIGURE 10.4 Insect pollinator screening-level risk assessment process for soil and seed treatment of systemic pesticides. Note that this flow chart may apply for trunk injection as well, as modalities of exposure of pollinators are similar as for soil/seed treatments. For trunk injection, however, further data are needed to appropriately describe the range of expected residue concentrations in nectar and pollen. As a consequence, no default value is currently available for a quantification of the risk (Boxes 3a and 3b). A compilation of available data could be made, with a particular attention to the corresponding injection protocols as it varies with the active substance involved and the tree. (For a color version, see the color plate section.)

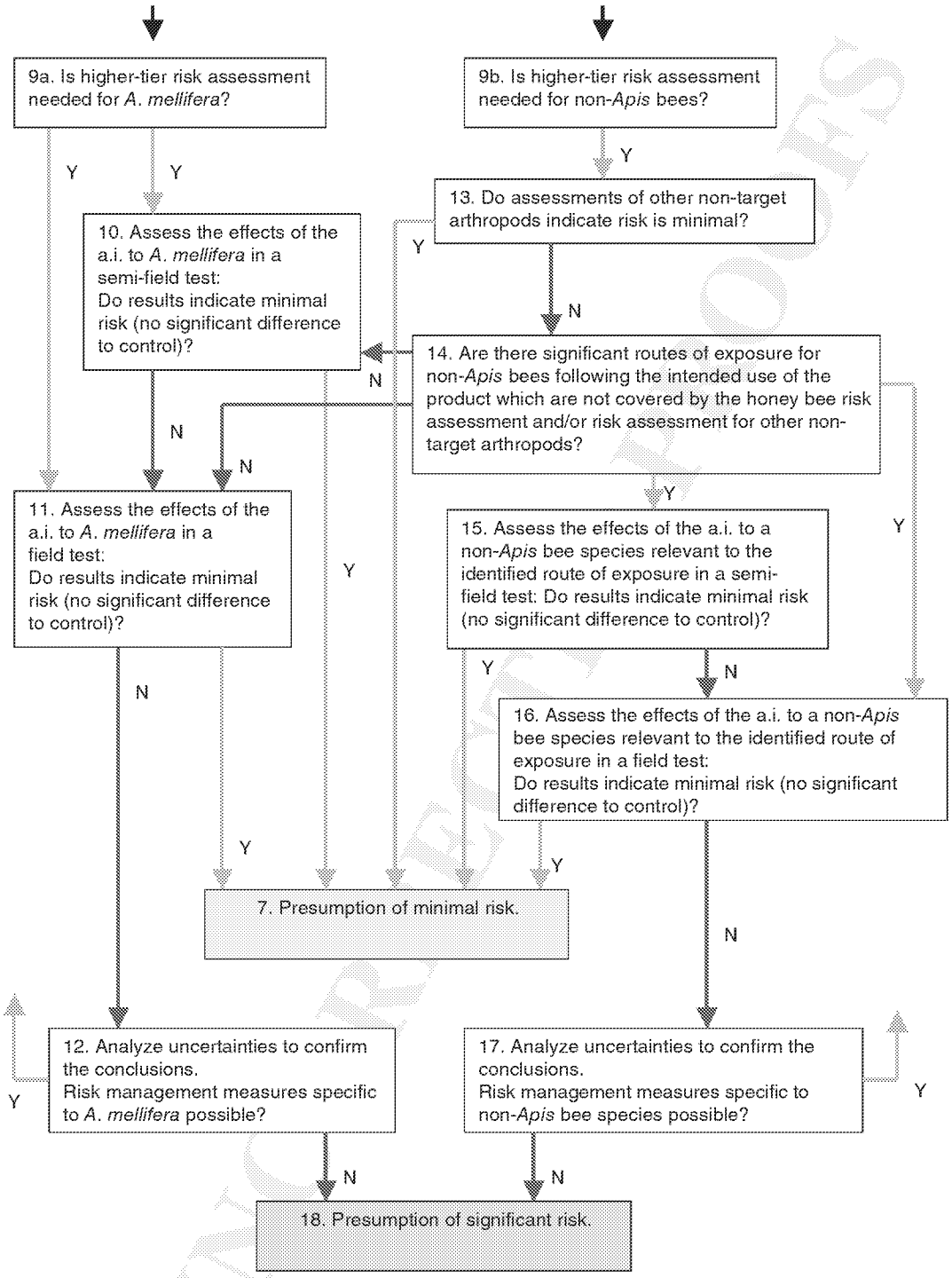

FIGURE 10.5 Higher tier (refined) risk assessment process for soil and seed treatment applied systemic pesticides. (For a color version, see the color plate section.)

to note that contact exposure to a systemic compound is also possible if it is applied as a spray application around or during the flowering period, for example, in the case of pre-bloom application. In this case, the reader may also find useful recommendations in the flowchart for soil/seed treatments.

Each box of these flowcharts is numbered and the nature of the data and reasoning behind each step of the process is provided below. As noted earlier, suitable level of concern (LOC) values (i.e., trigger values) for transitioning to higher levels of refinement are linked to risk management decisions and protection goals of individual regulatory authorities. The trigger values depicted in Figures 10.2 through 10.5 are generic. However, the more detailed but related risk assessment scheme in Appendix 6, which modifies the EPPO guidance (EPPO, 2010), contains some trigger values currently used in the European regulatory process (EC, 2010). As stated in other parts of this document, it is not the intent of this document, or SETAC, to recommend and/or support any particular trigger criteria but rather to emphasize the role that these values play in an efficient risk assessment process.

10.4 SPRAY APPLICATIONS

Figures 10.2 and 10.3 depict the risk assessment process for insect pollinators following the use of spray products. Each step (box) depicted in the flow chart is numbered and arrows depict the direction that should be followed in response to a "yes" or "no" answer. More details regarding each of the steps are provided below.

The risk assessment process begins by asking whether exposure is possible (**Box 2a**); if exposure is not possible, then there is a presumption of minimal risk (**Box 6**). For sprayed applications, the screening level considers the worse case exposure assumption of a direct overspray to plants where bees are actively foraging. Potential effects of the chemical thus result from the overall effects of the direct spray on foraging bees.

As depicted on the left-hand side of Figure 10.2, at the screening level, the potential risk to adult honey bees from spray applications is assessed through calculation of an HQ (**Box 3a**). The assessor calculates an HQ by dividing the theoretical exposure, that is, the application rate expressed in terms of weight per unit area (e.g., grams active ingredient/hectare) by the most sensitive acute median lethal dose to 50% of the organisms tested, that is, the (contact) LD50 value, derived from laboratory studies. If the HQ value passes a regulatory trigger value, then there may be a presumption of minimal risk to adult honey bees and the reviewer proceeds to assess possible impacts to non-*Apis* adults (**Box 4a**).

To evaluate potential risk to *larval* honey bees, the assessor calculates a TER by dividing the most sensitive No-Observed-Effect level (NOEL) from the honey bee larval toxicity test by the theoretical maximum concentration in pollen and nectar (**Box 3b**). While several test designs currently exist to assess effects to larvae, adoption of this step in a formal, regulatory process would require standardization of a particular test design. Possible test designs for lower tier laboratory-based studies with larvae are discussed in Chapter 8. If the TER value passes the trigger value, then a presumption of minimal risk to larval honey bees can be made and the reviewer proceeds to evaluate possible impacts on non-*Apis* larvae (**Box 4b**).

Default Exposure Estimates for Screening Level Analysis for Apis Larvae: Although a theoretical maximum concentration has been established by some regulatory authorities for systemic products (e.g., 1 mg/kg or ppm, EPPO, 2010) no such exposure model or theoretical maximum concentration level has been formally set for sprayed products. Pesticide residues resulting from direct overspray on food items for birds and mammals can be estimated using a residue per unit dose (RUD) approach favored by Hoerger and Kenaga (1972). The EPA terrestrial exposure model (T-REX) has been revised to include insect residue data that could represent reasonably conservative screening values (USEPA, 2012b). In the most recent guidance produced by the European Food Safety Authority (EFSA, 2009), a range of RUD values have been developed for different crops and food sources. Furthermore, the EPA toxicity of residues on foliage test may provide insight on the magnitude of residues on foliage following a particular application rate and the period of time these residues

remain toxic (USEPA, 2012a). Further research is necessary to both validate current screening exposure values used by regulatory authorities, as well as to develop RUD values, or other (screening) exposure models specific to pollinators.

The proposed risk assessment scheme also considers potential risks to non-*Apis* bees. At the screening level, risk to non-*Apis* bees is evaluated by employing effects data from honey bee acute oral/contact (LD50) studies (**Box 4a** depicting the calculation of an HQ for non-*Apis* adults), and chronic larval honey bee toxicity (NOEL) test data (**Box 4b** depicting the calculation of a TER for non-*Apis* larvae). In cases where Tier 1 (screening-level) data on *Apis* bees are not sufficient to conclude low risks to non-*Apis* bees (i.e., using a trigger value for *Apis* species modified with an appropriate safety factor to account for inter-species variation), then it may be concluded that the substance does not pass the screening step. In this case, data from non-target arthropods (NTA), typically required in the European registration process, could be considered (**Box 4a and 4b**) as they may provide useful information on the choice of non-*Apis* species to be further tested if potential risk cannot be excluded upon examination of the available NTA data. Participants in the Workshop agreed that NTA data could be utilized as it typically includes toxicity estimates for the predatory mite (*Typhlodromus pyri*) and the parasitic wasp (*Aphidius rhopalosiphi*). Refined risk estimates for non-*Apis* bees would then require development of adult oral and/or contact LD50 values for the relevant non-*Apis* species and an HQ (i.e, application rate/LD50) developed for adult bees (**Box 5a**). Similarly, where risk estimates do not meet trigger criteria for non-*Apis* bee larvae, then a NOEL for relevant non-*Apis* bees is necessary (**Box 5b**) to calculate a TER. As with toxicity estimates for adult non-*Apis* bees, toxicity test methods would have to be developed for larvae of relevant non-*Apis* bees. If risk estimates for either adult and/or larval non-*Apis* bees are within regulatory criteria, then minimal risk is presumed (**Box 6**); however, if not, then the reviewer should proceed to higher tier (refined) assessment methods depicted in Figure 10.3 or consider risk mitigation measures intended to reduce exposure (**Box 7**). As depicted in Figure 10.2, where risk mitigation measures are imposed, the reviewer should then re-evaluate whether exposure to adults (**Box 2a**) and/or larvae (**Box 2b**) has been sufficiently reduced to presume minimal risk. Again, if minimal risk cannot be presumed, the reviewer should proceed through the screen using the revised exposure numbers based on the proposed mitigation.

The proposed refined risk assessment for sprayed products depicted in Figure 10.3 begins by asking whether higher tier risk assessment is needed for honey bees (**Box 8a**) or for non-*Apis* bees (**Box 8b**). The screening level risk assessment is typically based on effects data on individual bees collected through laboratory studies. However, in refined risk assessments, the reviewer considers the results of semi-field and full field tests, which are typically conducted at the colony level rather than at the level of the individual bee. The refined risk assessment process therefore attempts to capture more realistic effects data as well as incorporating more refined estimates of exposure. For honey bees, effect estimates from semi-field studies (**Box 9**) or full field studies (**Box 10**) are used to determine whether maximum application rates result in effects. If minimal risk cannot be presumed from the results of semi-field studies, then the reviewer should consider full field studies where such studies can determine effects under more realistic test conditions (**Box 10**). In cases where full field studies do not result in risk estimates that are consistent with protection goals, then the reviewer should conduct an analysis of uncertainties associated with the review process and determine whether possible mitigation specific to honey bees has been adequately considered (**Box 11**). As in the screening-level assessment, the impact of mitigation measures should be considered through the refined risk assessment process to ensure that their result is inconsistent with protection goals. After such an analysis, if risk estimates still do not meet regulatory criteria, then there is a presumption of significant risks (**Box 17**).

In the case of non-*Apis* bees, the reviewer assesses potential risks via data on NTAs (**Box 12**) and determines whether there are actual significant routes of exposure which are not accounted for by the higher tier tests conducted using honey bees (**Box 13**) such as from contaminated nest material. If risk concerns to non-*Apis* bees cannot be minimized, higher tier effects testing discussed in Chapter 9 using non-*Apis* bees relevant to the specific potential route of exposure are then considered, possibly first through a semi-field test (**Box 14**)

with the option to extend the investigation to the full field level (**Box 15**). As with honey bees, the process and underlying assumptions/uncertainties associated with risk estimates should be carefully analyzed (**Box 16**) and the reviewer should consider possible mitigation measures specific to non-*Apis* bees. The potential effects of mitigation options must be considered at each of the steps within the refined process whether it is an *Apis* or non-*Apis* analysis. If after this analysis, estimates are considered reasonable and potential mitigation measures cannot reduce potential exposure and potential risks, then the reviewer must presume significant risk to the non-*Apis* species, under the proposed conditions of use.

10.5 SOIL AND SEED TREATMENT APPLICATIONS FOR SYSTEMIC SUBSTANCES

The screening-level and refined risk assessment processes for soil/seed treatment-applied pesticides are depicted in Figures 10.4 and 10.5, respectively. Pesticides used as soil treatment or soil treatments are conservatively assumed to be systemically distributed to plant tissues as the plant develops. Each step (box) depicted in the flow chart is numbered and arrows depict the direction that should be followed in response to a yes or no answer. More detail regarding each of the steps is provided below.

Please check the phrase "soil treatment or soil treatments" mentioned in the newly added sentence (as per Query reply) "Pesticides used as soil treatment or soil treatments are conservatively assumed to be systemically distributed to plant tissues as the plant develops" for clarity.

When evaluating potential acute risk to adult honey bees from soil or seed treatments[4] with systemic compounds, the assessor first asks whether exposure is possible to the adult (**Box 2a**) or immature stages (**Box 2b**) via systemic translocation of residues in plant material. If exposure to honey bee adults is considered likely, the review calculates a TER (**Box 3a**) using either an acute oral or contact LD50 value for honey bee adults. In Europe, a Tier 1 TER is estimated by dividing a screening exposure estimate by the screening level hazard value. Currently, EPPO has a proposed conservative default exposure value of 1 mg a.i./kg, relies on the default maximum concentration estimated in pollen and/or nectar from residues in whole plants, which for use with soil and seed treatments. If the risk estimate for the adult honey bees does not meet the regulatory criterion for low risk, then the reviewer should proceed to higher tier risk assessment (options to proceed with a 10-day adult test (**Box 4a**), or more refined studies) or consider risk mitigation measures and reassess (**Box 8**). If the TER value for the adult honey bee meets the regulatory criterion for low risk, then the reviewer proceeds to evaluate potential impacts on non-*Apis* adults (**Box 5a**). Here the assessor may consider data on NTAs. Where risk assessments for non-*Apis* bees do not meet the regulatory criterion for low risk (i.e., meets the regulatory criterion for low risk to *Apis* by a wide margin), then acute oral/contact LD50 values should be developed for non-*Apis* bees and a TER calculated (**Box 6a**). As with honey bees, if the risk estimate does meet the regulatory criterion for low risk, then the reviewer should proceed to higher tier (refined) risk assessment (semi-field or field study) or consider risk mitigation measures and reassess (**Box 8**).

For larval assessments, the same process as that discussed for spray applications is followed (Boxes **3b**, **4b**, and **5b** of Figure 10.4). Additionally, the same process for higher tier (refined) risk assessment is used as discussed for spray applications. Participants of the Workshop noted the lack of information on potential exposure (nectar and pollen) related to trunk injection; and that further data are needed in this area (see Chapter 13). In the meantime, participants of the Workshop recommended that potential (screening) risks from trunk injection be estimated in the same manner as soil and seed scenarios.

As discussed previously, risk assessment is intended to be an iterative process. At a screening level, when risk estimates do not meet decision criteria (i.e., where a presumption of minimal risk cannot be made), the conditions under which the estimated risks occur should be more closely examined. More detailed fate considerations (such as degradation), or use considerations (such as timing of application, or application intervals) should be considered before additional testing is required.

[4] Although not specifically discussed at the Workshop, treatments with systemic compounds can include tree trunk injections as well.

10.6 SCREENING-LEVEL RISK ASSESSMENTS (TIER 1)

As noted, ecological risk assessments typically follow a tiered process (depicted in Figure 10.1). Substances move through lower tiers to higher tiers when the information indicates potential risk cannot be excluded. The first tier of that process is the screening-level assessment, which is intended to effectively and rapidly:

- exclude substances of low risk concern from entering into resource intensive higher tier risk assessment; and
- identify substances for which a potential risk to bees cannot be excluded and for which a higher tier risk assessment is needed.

The screening-level assessment should allow for the most efficient allocation of resources and minimize the number of substances forwarded for higher tier evaluation while still identifying substances of potential risk to bees. An efficient screening step in the risk assessment process is essential as it optimizes the success in achieving protection goals. At a screening level, the intent is then to use an appropriately sensitive species that is suitable to ensure that protection goals will be met. In this context, in designing the risk assessment process, participants proposed the *honey bee* as a reasonable surrogate for both *Apis* and non-*Apis* bees at a screening level for evaluating acute toxicity to adults. The reasons for this are:

- the biology and availability of *A. mellifera* makes it well-suited and lends itself to testing and analysis;
- the relative sensitivity of the honey bee compared to non-*Apis* species (based on available data)
- tiered toxicity test guidelines are widely available for *A. mellifera*; and
- conducting and interpreting the results of these tests does not require specialized backgrounds and/or conditions.

As illustrated in the flow chart depicted in Figure 10.1, the screening step most often relies on the calculation of risk estimates, termed RQ, HQ, or TER. These risk estimates are compared to numerical regulatory decision criteria, termed an "LOC" or "trigger criterion." An LOC is a value against which a risk estimate is compared. It is intended to be protective in that it typically accounts for uncertainties related to intra- and inter-species variation in sensitivity, extrapolation of short-term toxicity to long-term effects, and extrapolation of laboratory results to the field.

Depending upon the type of risk estimate used (RQ or TER), if the estimate is above or below the LOC, then a determination of minimal risk is presumed, or whether additional refinements are necessary. For example, if screening-level risk estimate results in a TER (where the effects estimate is divided by the exposure estimate) that exceeds the LOC, then minimal risk is presumed (i.e., if TER > LOC = minimal risk is presumed); conversely, if the TER does not exceed the trigger value, then minimal risk cannot be presumed, and a higher tier risk assessment may be needed. The RQ is the reciprocal of the TER in that the exposure estimate is divided by the effects estimate; therefore, the RQ value is interpreted opposite to the way in which the TER is interpreted, that is, if the RQ exceeds a trigger value, then minimal risk is not presumed and a higher tiered risk assessment may be needed. If the RQ value is greater than the LOC (or trigger value), then minimal risk cannot be presumed.

10.7 FACTORS LIMITING CERTAINTY IN SCREENING ASSESSMENTS

Screening-level assessments are typically based on conservative assumptions regarding both exposure and effects. For example, at a screening level assessment for honey bees, the EPPO system does not account

for good practices such as avoiding spray application during foraging times but conversely, not all routes of potential exposure are reflected. Given all the potential variables to consider, the participants of the Workshop believed that the proposed screening level analysis is conservative and protective for other potential routes of exposure.

Similarly, although mortality is the primary effect reported and used to generate LD50 values in acute toxicity tests, adverse effects on behavior are also reported. As discussed in earlier chapters, the extent to which sublethal effects occur and whether they ultimately affect assessment endpoints such as impaired survival, growth, and reproduction at the colony level remains an uncertainty for many compounds. However, since effects on behavior are frequently, but not exclusively, associated with insecticides or acaricides which will also potentially affect acute survival, the majority of these compounds will be subject to higher tier risk assessment where the sublethal effects will be more thoroughly evaluated. In addition, other information presented in the data profile of a compound (such as mode of action, route of uptake, toxicity, and effects on other types of terrestrial arthropods) should always be examined (EPPO, 2010), and integrated with the findings of the screening step as part of the overall risk assessment for honey and non-*Apis* bees.

The capacity of the screening-level assessment to properly screen substances of low likelihood of adverse effects from substances for which further assessment is necessary has been evaluated through a review of the honey bee kill incidents recorded in the United Kingdom survey network Wildlife Incident Investigation Scheme (WIIS) (Mineau et al., 2008). The Mineau et al., 2008 analysis supports the utility and efficacy of the Tier 1 screening methodology, provided that considerations on the mode of action and use patterns are also kept in mind, as for any risk assessment process.

10.8 REFINEMENT OPTIONS FOR SCREENING-LEVEL RISK ASSESSMENT

If the results of a screening-level assessment indicate that a minimal risk cannot be concluded, the process moves to a series of refinements in exposure and/or effects data (see Figures 10.2 through 10.5). There are a number of options to further refine a risk assessment through a more in-depth description/characterization of exposure and/or of effects. These options are described, regarding their possible methodologies, in previous chapters. As refinements progress, different TERs and RQs are developed.

In the deterministic risk assessment approach, the primary outcome of the (Tier 1) risk characterization is the calculation of the RQ, or the TER depending on the country/region where the assessment is being performed. Both the RQ and the TER are single number (point) risk estimates. In reality, risk is more complex and therefore, a single point estimate can be misleading. As a consequence, the assessor should characterize the RQ or TER with a description of the uncertainties, assumptions, strengths, and limitations associated with the risk estimate. These sources of variability and uncertainty should be discussed during characterization of the exposure and effects and should include refinement options used in ultimately determining the RQ or TER. At the higher levels of refinement (e.g., semi-field and field tests), the level of impact is directly measured in experiments that are intended to reproduce the operational conditions of the subject pesticide product. In this case, TER and RQ values are no longer calculated.

Exposure is the first component of the risk to be examined to determine whether a risk assessment is needed, and the first to be explored to refine a potential risk. As a guide for proceeding through the levels of refinement, Table 10.3 provides a summary of the relative importance of different exposure routes for *Apis* and non-*Apis* bees. The main exposure routes identified for evaluation in the screening-level assessment are oral intake of nectar and pollen, and contact exposure. While not all exposure routes are included in the screening-level (Tier 1) risk assessment (e.g., wax and drinking water are not evaluated at Tier 1); and, direct overspray is considered as the worst case (high-end) exposure, it is important for the assessor to consider additional exposure routes for higher tier risk assessment purposes (Table 10.3 presents potential exposure routes for different bees).

TABLE 10.3
Likelihood of Exposure to *Apis* and Non-*Apis* Bees from Various Routes

	Apis		Non-*Apis*	
Exposure	**Adults**	**Larvae**	**Adults**	**Larvae**
Nectar	+ + + [1]	+	+ to + + +	+
Pollen	+ to + + +	+ + [2]	+ to + + +	+ + to + + +
Water[a]	+ to + +	+ [3]	+	+
Nesting material	+ [4]	+ [4]	+ to + + + [4, 5]	+ to + + + [6, 7, 8]
Exposure to soil	±	–	– to + + +	– to + + +
Foliar residues (Contact and direct spray)	+ + +	–	+ + +	– to + + +
Direct spray	+ + + [9]	–	+ + + [9]	–

[a]Collect water for cooling (evaporative cooling; take up into crop, regurgitate it, and flap wings to distribute) and honey production.
[1]Particularly for nurse bees; [2]bee bread; [3]provided by nurse bees; [4]wax; [5]leaves and soil for cement; [6]leafcutting bees; [7]soil used to cap cells; [8]exposure to soil; [9]at flowering.

Other insects may experience these exposure routes and testing methods are available for these species and field data may be available. As an example, parasitoid species also feed on nectar, such as the predatory mite *T. pyri,* or the ladybird beetle *Coccinella septempuctata* feeds on pollen. The predatory and parasitoid coleopteran *Aleochara bilineata* is a soil dweller at the adult stage. Therefore, review of these data when available may be useful in determining the major exposure routes to be investigated in a risk assessment for pollinators.

10.8.1 Refinement Options for Spray Applications

10.8.1.1 *Apis* Adults

If the HQ for adult *Apis* exceeds the LOC in the screening-level (Tier 1) assessment, then further information is required. Refinements can be made for exposure and/or effects, depending upon the profile of the active substance and its residues.

For spray application, an option for refining exposure estimates is to move from the screening-level default values to product-specific field modeling or measurement data to better quantify exposure. If an application during flowering cannot be excluded, this option may have several levels of refinement such as consideration of the interval between application and flowering and the expected level of residues to which bees could be exposed, for either modeled or measured estimates of refined exposure. Measurements of actual exposure may be achieved by the use of existing residue data, for example, magnitude of residue, or by implementing tunnel and/or field residue studies to estimate the level of exposure in treated crops and considering different modalities for the period of treatment.

While most semi- and full-field toxicity tests generate data on both exposure and effects, they may also be pursued with an exclusive aim of providing realistic exposure estimates. In this case, it is important that data generated from the field test is recorded so that it may be directly compared to the ecotoxicity data (i.e., the results and endpoints are expressed in the same units and represent comparable measures of exposure).

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With respect to residue concentrations in nectar and pollen (or foliage where appropriate), the reviewer should consider the 90th percentile of measured concentrations as a conservative measure of exposure. However the decision to use a 90th percentile or other value ultimately depends on the dataset. If data are derived from only a single test on one crop, then a specified percentile, for example, 90th percentile, should be sufficiently vetted to reflect the uncertainty and variability as is frequently done in support of probabilistic approaches. If several trials have been undertaken, or data are derived for several crops, then a mean or a lower percentile may be more appropriate and would achieve the same level of protection. The selection of a particular crop for the evaluation of residues must consider whether the resulting data are sufficiently conservative to enable those data to serve as a surrogate for other uses.

The initial test to measure the effect of a compound is a lethality test consistent with relevant life stage and exposure route (e.g., oral LD50, or larval toxicity test). As effects tests become more refined, they incorporate more environmentally realistic conditions and begin to reflect both intrinsic toxicity and potential-enhancing/compensatory effects, related to environmental conditions.

To further refine the toxicity endpoint, additional *Apis* studies that could be relevant for the adult life stage include:

- 10-day feeding study (adult survival);
- toxicity of residues on foliage study;
- semi-field data; and
- field data.

A description of the studies that may be appropriate is found in Chapter 9; these studies are discussed briefly below.

The 10-day adult study is an extension of the standard laboratory oral exposure method (OECD 215). The test exposes adult bees for a period of 10 days and measures lethal effects after ingestion of product over the entire test duration. A NOEL is derived that may be used similarly as a LD50 in RQ calculations. Because this test only addresses oral exposure, it is not sufficient to address the uncertainties associated with sprayed compounds and is actually considered to be useful when refining estimates of effects for systemic soil/seed treatments. Currently there is no internationally recognized guideline for the 10-day feeding study nor for the larval toxicity testing in the laboratory; therefore, these tests need to be developed and validated before formal inclusion into a regulatory risk assessment scheme. The endpoint from a 10-day feeding study could be compared to either the default (screening-level) exposure concentration, or to refined exposure concentrations based on field measurements, both expressed in mg a.i./kg.

The EPA foliar residue toxicity study is more representative of the conditions of exposure for bees after a spray event. This study is designed to evaluate the effects from exposure to dry and aged residues (3, 6 and 24 hours) and thus provide information on the level of bioavailability and length of residual hazard of the substance.

As discussed in Chapter 9, semi-field, and field studies reproduce more closely the conditions of exposure of bees in a treated crop. The test provides information on colony health based on bee survival and development related to actual field application parameters. Semi-field and field tests can be undertaken with pollinator-attractive crops treated at flowering (e.g., *Phacelia*), and/or pursued with the actual target crop when a treatment at flowering cannot be excluded. Semi-field and field tests can also provide additional information to refine an assessment such as information on potential exposure outside the flowering period of the crop, or through spray drift onto flowers in vegetated areas, or onto flowering weeds within the crop (e.g., in orchards). Finally semi-field and field tests may allow the evaluation of the efficacy of certain risk mitigation measures to limit exposure such as reduced application rates, or modifying application intervals.

10.8.1.2 *Apis* Larvae

As for the adults, an option for refinement of exposure is to move from the screening-level default values (e.g., application rate or default consumption rate), to product-specific field modeling or actual measured residues (e.g., in pollen and nectar, honey or beebread) to better quantify exposure of larvae. The same considerations with regard to the generation and use of these data apply.

Additional *Apis* studies that could be relevant for the larval or immature life stages include:

- brood-feeding studies (brood development[5]);
- semi-field studies; and
- field studies.

The brood-feeding study aims at evaluating the effects on the development of the honey bee to derive a no-observed-effect concentration (NOEC). This NOEC can then be compared to either default (screening-level) concentration estimates or to refined concentrations based on field measurements.

The semi-field and field tests are similar with respect to measurement of effects on adults and both can provide information on colony health and brood development. As discussed elsewhere, field studies typically do not lend themselves to producing a dose–response relationship (i.e., a NOEC or LOEC) due to scale and logistical reasons. Consequently, the assessor must evaluate whether the study results indicate that a minimum level of risk exists (for example, no significant (or limited) difference between test and control plots). Increased levels of refinement toward characterizing effects beyond the laboratory and semi-field may involve assessing impacts of the formulated product in full field tests. Further discussion and guidance on semi-field, and field tests can be found in Chapter 9, and discussion and guidance on brood tests can be found in Chapter 8.

10.8.1.3 Non-*Apis* Adults

Non-*Apis* bees may differ from honey bees in their exposure and sensitivity to plant protection products. Most non-*Apis* bees are solitary, with single females that forage for pollen and nectar to feed their offspring, construct their nests, and lay eggs (see introduction to non-*Apis* biology). The death of a foraging female implies the cessation of her reproduction (Tasei, 2002). In comparison, when a (honey bee) colony loses female workers, the loss may be compensated by the colony, for example, by engaging inactive workers (Robinson, 1992) or through reduced foraging age (Winston and Fergusson, 1985), so the colony may continue to develop as a viable unit. For bumble bees some colony recovery is also possible (Schmid-Hempel and Heeb, 1991). However, the death of the bumble bee queen in the spring signifies the death of the potential colony that would be formed (Thompson and Hunt, 1999).

In comparison to honey bees, the life-history traits of non-*Apis* bees such as sociality and nesting behavior result in a greater importance of certain exposure routes. For example, alfalfa leafcutting bees (*Megachile rotundata*) may be more exposed to foliar residues (George and Rincker, 1982), ground-nesting bees to soil residues and larvae to pollen residues. These differences mean that representatives of the main non-*Apis* groups for which we have sufficient knowledge should be considered for higher tier testing of a plant protection product for bees when a risk cannot be excluded. Where non-*Apis* species are chosen for higher tier evaluation they should be amenable to experimentation, provide reliable and reproducible results, and the methods should comply with internationally recognized and validated guidelines (e.g., OECD test guidelines). The exact choice of species may be based on the proposed use of the product and on regional

[5] For example the method of Oomen et al. (1992).

(species) considerations; however, it should be possible to extrapolate from "standard" species (e.g., *Bombus* sp.) to reduce the need for unnecessary testing.

Participants of the Workshop proposed that higher tier testing could be conducted with social non-*Apis* bees from the tribes Bombini and Meliponini and solitary bees that are ground-nesting and cavity-nesting (Table 10.5). While techniques exist for both laboratory and field/semi-field tests for Bombini spp. (*B. terrestris* and *B. impatiens*) standardization is needed (for review on *Bombus* spp. see Van der Steen, 2001). Similar tests are in development for Meliponini species. Sufficient knowledge exists of the ecology of the Bombini and Meliponini tribes to be able to predict the main exposure routes (see Chapter 7). For cavity-nesting solitary bees (*Osmia lignaria* and *M. rotundata*), laboratory and field/semi-field tests have already been successfully developed (Alston et al., 2007; Abbott et al., 2008; Ladurner et al., 2008). For ground-nesting bees, while primary exposure routes can be predicted, there are not yet the techniques to perform standardized tests on them in the laboratory or the field. Until such techniques are available, the solitary cavity-nesting bees may sufficiently represent "solitary non-*Apis*" as a group, taking into account that for ground-nesting species, soil residues may play a more important route of exposure. Note, however, that even for Bombini and Meliponini tribes no validated or internationally recognized test protocols exist which currently limits their inclusion into a risk assessment scheme at this point in time and further research is needed.

Exposure Similar to the refinement process for adult honey bees, the option for refinement of exposure to adult non-*Apis* bees is to move from the screening-level default values to product-specific field modeling or measurement data to better quantify exposure of non-*Apis* larvae. Table 10.3 provides further guidance on the specific conditions of exposure for non-*Apis* species. The same considerations with regard to the generation and use of these data apply.

Effects As discussed previously, at a screening level in the proposed risk assessment scheme, the adult *A. mellifera* is used as a surrogate for non-*Apis* species. To take into account interspecies variation and the different life-history characteristics a safety factor may be built into the LOC for *Apis* to non-*Apis* (participants of the Workshop considered a 10× factor conservative). Then, as illustrated in the flow chart, if the HQ is less than the adjusted non-*Apis* LOC, then risk is presumed to be low for non-*Apis* species; and, where it is not, further refinement of the ecotoxicity data may be undertaken.

When available, NTA data may be considered at this stage, as it may provide relevant information on effects (and route-specific exposure) to non-*Apis* species see Table 10.4.

The nectar-feeding parasitoid *A. rhopalosiphi* and the soil-dwelling beetle *A. bilineata* are among the most sensitive of the NTAs tested under the European ESCORT scheme (Candolfi et al., 2001). Adult parasitoids such as *Aphidius* also feed on nectar, which makes it a good NTA representative for exposure conditions of pollinating species. Similarly, approximately 70% of non-*Apis* bees are ground-nesting (Michener, 2000) and the ground-dwelling beetle *A. bilineata*, is tested for sensitivity to plant protection products through sand/soil under the European ESCORT scheme, such that data from its contact toxicity tests may be considered informative for ground nesting bees. In the cases where a refined risk assessment has been triggered for non-*Apis* adults, the dataset developed in the European process may contain information on up to 8–10 species in the laboratory and more when semi-field/field testing have to be undertaken for refined risk assessment purposes (Candolfi et al., 2001) (Table 10.4). In these cases, inventories of the species identified in the crops tested may also be useful information in evaluating whether a particular concern is raised for non-*Apis* species which would need to be investigated further. Additional work is needed to understand the relative sensitivity of NTAs typically used in toxicity testing to non-*Apis* bees for which they may be used as surrogates.

If relevant NTA data cannot be found, then the assessor may consider selection of an appropriate non-*Apis* species for use in acute laboratory testing (Table 8.3, see Chapter 8) and data from residue studies and field measurements (i.e., pollen, nectar, foliage and soil) can inform study design with respect to exposure for

TABLE 10.4
Testing Methodologies Developed for the Risk Assessment to Non-Target Arthropods Developed in the European Process of Evaluation of Pesticides

Testing Scale	Species (and Stages Tested)
Tier 1 laboratory: artificial substrate	*Aphidius rhopalosiphi* (adults + life cycle[a]) *Typhlodromus pyri* (protonymphs + life cycle[a])
Tier 2 (extended) laboratory: natural substrate	*Aleochara bilineata* (adults + life cycle[a]) *A. rhopalosiphi* (adults + life cycle[a]) *Chrysoperla carnea* (larvae + life cycle[a]) *Coccinella septempunctata* (larvae + life cycle[a]) *Orius laevigatus* (nymphs + life cycle[a]) *Pardosa* sp. (adults) *Poecilus cupreus* (adults) *Trichogramma cacoeciae* (adults + life cycle[a])
Semi-field	*P. cupreus* (adults) Methods can be adapted for many species
Field	Arthropods (populations and communities)

Source: Data from Candolfi et al., 2001.
[a]Studies purporting to examine the life cycle of species may focus on a particular aspect of the life cycle and may not include the entire life cycle.

non-*Apis* (see also Chapter 7). For example, a plant protection product with high foliar residues would suggest that higher tier testing should be performed on alfalfa leafcutting bees (*M. rotundata*) if such bees will visit the crop to harvest nesting material and exposure may occur.

Alternatively, as shown in the flow charts (Figures 10.2, 10.3, 10.4, and 10.5), non-*Apis*-specific test data for adult contact or oral toxicity can be generated. These data are likely to be in the form of an LD50 (μg/bee), to be used in developing an HQ similar to that for adult *Apis*. For the assessment criteria to be met, the HQ must not exceed the LOC (trigger value); if it does not exceed a concern, the assessment need not proceed further. Issues of LOC (triggers) and safety factors (such as intra-species variation) may be further discussed by the respective regulatory authorities.

Refinement of effects data beyond the laboratory and semi-field/field may involve assessing impacts of the formulated product. Guidance on the types of tests may be found in Chapter 8. The field or semi-field tests will monitor behavior and quantify bee mortality and fecundity of one or several selected non-*Apis* species likely to be encountered in the crops to be treated with the product. (see Chapter 8, Hazard, Field for methods and advantages of field tests on non-*Apis* bees). Table 10.5 at the end of this section highlights the availability of laboratory and field tests for representative groups of social and solitary non-*Apis* bees.

Risk Characterization (Estimation) For both *Apis* and non-*Apis* assessments, when higher level field data are developed, the results are not expected to be applied in a TER and/or quotient context, but may be used directly in the risk assessment. Again, mitigation of potential risk remains as an important pathway to meeting protection goals whether at the screening or higher tier steps of the analysis. Risk characterization will depend upon the data generated and refinements therein. Below is a brief discussion of refinements to input studies.

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TABLE 10.5
Available Laboratory and Field Tests with Representative Groups of Solitary and Social Non-*Apis* Bees

		Solitary		Social	
Study Type		**Tunnel-nesting (Tube, Wood)**	**Ground-nesting**	**Bombini (Bumble Bees)**	**Meliponini (Stingless Bees)**
Laboratory	Adult	Zone: temperate north *Megachile rotundata* (Huntzinger et al., 2008; Scott-Dupree et al., 2009) *Osmia lignaria* (Ladurner et al., 2005; Scott-Dupree et al., 2009) Zone: tropics *Xylocopa* spp. (tests in development)	Zone: temperate north *Nomia melanderi* (Johansen et al., 1984; Mayer et al., 1998)	Zone: temperate north *Bombus terrestris* (Thompson, 2001) *Bombus impatiens* (Scott-Dupree et al., 2009; Gradish et al., 2011b[a])	Zone: tropics several species, and tests in development (Macieira and Hebling-Beraldo, 1989; Valdovinos-Nunez et al., 2009)
	Larva	Zone: temperate north *M. rotundata* (Peach et al., 1995; Gradish et al., 2011a; Hodgson et al., 2011) *O. lignaria* (Abbott et al., 2008) Zone: tropics *Xylocopa* spp. (tests in development)		Zone: temperate north *B. terrestris* (Thompson, 2001) *B. impatiens* Gradish et al., 2010; Gradish et al., 2011b[a])	Zone: tropics (tests in development)
Field	Semi-field	Zone: temperate north *M. rotundata* (Johansen et al., 1984; Tasei et al., 1988; Mayer and Lunden, 1999) *Osmia bicornis* (Konrad et al., 2008) *O. lignaria* (Ladurner et al., 2008)		Zone: temperate north *B. terrestris* (Tasei et al., 2001) *B. impatiens* (Gels et al., 2002)	Zone: tropics (tests in development)
	Field	Zone: temperate north *M. rotundata* (Torchio, 1983) *O. lignaria*	Limited availability of tested species *Nomia melanderi* (Mayer et al., 1998)	Zone: temperate north *B. terrestris* (Tasei et al., 2001) *B. impatiens*	Zone: tropics (tests in development)
Exposure (pollen, nectar, foliar, soil)		Can be developed. For pollen provisions in the field, see Abbott et al. 2008 For foliar residues, see George and Rincker, 1982		For pollen, see Morandin et al. 2005	

[a]Needs standardized guidelines of currently used lab bioassay and microcolony assays.

UNCORRECTED PROOFS

10.8.1.4 Non-*Apis* Larvae

Exposure A general description of exposure sources for non-*Apis* species (immature stages) is provided in Table 10.3. Where honey bee larvae are exposed primarily in larval food (which is processed pollen) this should be considered when generating a refined (exposure) analysis for non-*Apis* species. For example, pollen sampled in the field or from loads taken at the hive entrance (pollen traps) or from forager bees directly may represent concentrations found in unprocessed food sources. Concentrations of residues from pollen sampled from within hive food stores or from larval cells could be more relevant to honey bee larvae.

Non-*Apis* larvae may also be exposed through contact with the pollen and nectar food provision in the nest. In addition, the larvae of ground-nesting bees and cavity-nesting bees which separate their nest cells with soil (e.g., *O. lignaria*) may come into contact with soil applied plant protection products. Similarly, the larvae of leafcutting bees may come into contact with a plant protection product through residues on the foliage used to construct its nest (see Chapter 7, Exposure). Non-*Apis* species thus have various sources of exposure (e.g., treated soil or nesting material). Refining potential exposure estimates to non-*Apis* bees to account for the different exposure sources would be difficult to achieve in a specific exposure test. In this case, it would be more appropriate to refine potential exposure and risk through a semi-field or field study (see Chapter 9).

Effects As discussed earlier, honey bee larvae are proposed as a surrogate for non-*Apis* larvae as there is currently no formal guideline established for testing non-*Apis* larvae.

As the assessor moves through the proposed process, they may consider NTA data, if available, which may provide relevant information to refine potential risk to non-*Apis* species (Candolfi et al., 2001). These tests measure a wide range of endpoints including juvenile and adult survival, fecundity, or larval development depending on the species being tested (Table 10.4). The NTA tests are frequently designed to detect relatively small changes in sublethal endpoints; therefore, an understanding of an application rate that may result in low impact on growth and/or fecundity or other sublethal parameters may be derived. Beyond laboratory tests, refining an understanding of potential effects to non-*Apis* larvae may involve field tests with formulated products (see Chapter 9). While field and semi-field tests have not been specifically developed for ground nesting bees, monitoring of cavity-nesting bees through field or semi-field tests may provide information on some of the larval exposure routes that are unique to non-*Apis* species. Table 10.5 at the end of this section highlights the availability of laboratory and field tests for representative groups of social and solitary non-*Apis* bees.

Risk Characterization (Estimation) If effects data on non-*Apis* larvae have been generated and provide a NOEC, then this value could be used as in TER calculation. Both default and refined exposure estimates may also be used in TER calculation. As noted in the flow charts, should this assessment indicate risks that are not consistent with protection goals, then, either mitigation measures may be considered or the assessment may proceed to further refinement.

Again, when data are generated from field tests, it is not expected that the results are conveyed in a TER (quotient-based) context, but rather incorporated directly into a risk assessment.

10.8.2 Soil or Seed Treatment Application for Systemic Substances (also including trunk injection)

10.8.2.1 Exposure—*Apis* and Non-*Apis*

While there are differences in the screening-level assessment for calculation of HQs/TERs between sprayed pesticides and systemic substances, the general approach to refining the risk assessment for systemic applications is largely similar to that for spray applications. The primary difference is that for systemic chemistries,

exposure levels via contact are largely below that which may be encountered via an oral route of exposure. Table 10.3 should be consulted for exposure routes specific to non-*Apis* bees. For example, for systemic compounds, leafcutter bees may be exposed orally through the foliage used to build their nests. The most appropriate way to explore this further is through simulating exposure conditions in a semi-field or a field test (see Chapter 9).

As stated earlier, for trunk injection, further data are needed to appropriately describe the range of expected residue concentrations in nectar and pollen that may be used in a risk estimate for this application method. In the future, a compilation of available data could be made, with particular attention to the corresponding injection protocol as it varies with active ingredient and tree species.

10.8.2.2 Effect—Adult *Apis*

If risk cannot be excluded at the screening-level assessment, then a Tier 2 assessment, based on the 10-d NOEL for young adult honey bees, can be conducted. The 10-day test is an appropriate measure to refine the acute effects endpoint employed in the Tier 1 assessment (i.e., oral LD50). The 10-day test may be run based on the default maximum concentration estimated in pollen and/or nectar, or on refined measured values, if these are available (see section 10.8 for more details on the options). In this case, if the TER value exceeds triggers, then one may reach a presumption of low risk to adult honey bees from soil/seed applications. If viable exposure routes exists for the immature stages of either honey bees or non-*Apis* species, (e.g., through contaminated pollen or beebread), then the approaches for refinement to soil/seed scenarios are similar as that for spray treatments (see Chapter 9). For higher tier testing (semi-field and field testing) protocols may be adapted to reflect crops grown from coated seeds or to products applied on/to soil, or for trunk injection. These tests may include monitoring of effects at sowing if measurements from potential exposure via seed dust (if it cannot be excluded or mitigated), or measurements of potential exposure to non-*Apis* species that might frequent the soil.

10.8.2.3 Risk Characterization (Estimation)

Similar principles as for spray application do apply for soil/seed treatments and trunk injection.

10.9 CONCLUSIONS ON THE RISKS AND RECOMMENDATIONS

Concluding a risk assessment is probably the step that best reflects how case-related the risk assessment process can be. Conclusions could be very brief and simply indicate that under the assessment that was conducted (i.e., whether it was screening level or a higher tiered assessment) the use of the product meets the protection goals of the respective regulatory authority. However, where a refined risk assessment was triggered, there is a need to clearly express the following information in the conclusions:

- what concerns were identified at the screening step;
- whether/what concerns were identified in higher tier assessments;
- whether results of the higher tier assessment, addressed potential risk concerns;
- whether/which mitigation measure were considered at different levels of analysis, and whether the mitigation measures reduced potential risks to an acceptable level;
- whether, despite higher tier analysis, all available lines of evidence, and consideration of mitigation measures, potential risks remain; and
- remaining uncertainties (if any) in the risk assessment.

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Risk assessment conclusions should give particular emphasis to the four following areas which are essential in providing appropriate information to risk managers for decision making. These are:

- the appropriateness of the available data to assess potential risks posed by the subject compound, or product;
- defining the use parameters required in order that the protection goals can be met;
- characterization of any potential risks, including remaining uncertainties resulting from a lack of data or deficiencies in the existing data; and
- where refined risk analysis indicates risk, characterization should be provided regarding the growth, reproduction or survival of the organism (colony/population) and possible interactions with plants and ultimately with stated protection goals.

Risk assessment conclusions should characterize the possibility of risk based on the available lines of information (data, monitoring information, incidents, etc.). Characterization should include discussion of potential risk to any specific life stages or castes. In certain cases, exposure considerations should focus on gathering more refined data such as:

- characterizing spray drift onto adjacent crops/vegetation that are attractive to bees; and
- characterizing exposure to residues that could reach pollen/nectar of the crop for pre-flowering applications of systemic compounds, and of mobilization of soil residues in rotational crops (where relevant).

The risk assessment should be able to address the meaning of effects, for example, a temporary increase in the mortality of foragers, avoidance of a treated crop over the first days post treatment, etc. Field, and in some cases semi-field, studies may allow for the monitoring of colonies/populations over long periods and measurement endpoints may be available to address these concerns. Unresolved issues regarding time scale (temporal) or spatial scale could also be addressed through modeling tools when sufficiently developed.[6] Where uncertainties are related to "borderline" or "minor" effects and do not strictly compromise the protection goals, they may be appropriately addressed by implementing a monitoring study. The advantage of monitoring in this respect is to verify that protection goals will be met under conditions of agricultural practice in the real environment without any effort to control other stress factors.

If a decision is made not to authorize a use, then it must be based on the evidence that protection goals for a particular product cannot be met. The inability to meet protection goals implies that, based on the available lines of evidence and higher tiered analysis, neither exposure nor hazard can be reduced or avoided, and resulting risks may compromise protection goals. It is the responsibility of both the risk assessor and risk manager to discuss the conditions of the assessment and explore mitigation options, if these are warranted. Both the assessor and manager should consider whether information exists that would determine whether all options to refine or mitigate potential risks have been explored before a final decision is reached.

10.10 RECOMMENDING RISK MITIGATION MEASURES

Risk mitigation measures mainly aim at reducing the risks through a reduction of exposure. In principle, mitigation may be considered at any stage of the assessment process, such as prohibiting application during bloom. However, certain measures aiming at reducing the level of exposure/residues in relation to effect

[6] Modeling tools have been successfully developed in other areas of ecotoxicology for that purpose.

threshold (NOEL), are more effectively considered during higher tier testing, such as reduced application rates or increased application intervals. Dedicated field testing may be useful when dealing with the product-specific measures. The decision to consider mitigation measures at any step of the process involves issues of product efficacy, as well as national policies. A fuller address of mitigation measures is found in Chapter 13.

10.11 ADDITIONAL TOOLS IN SUPPORT OF RISK ASSESSMENT AND TO INFORM RISK MANAGEMENT

Tools that may help to better interpret data (e.g., statistical and mathematical tools) should be used, particularly when higher tier data have been generated. In addition to these tools which now often enter into the usual package of risk assessment, modeling, and landscape management approaches are possibly the most promising ones to further support both risk assessment and risk mitigation provided these tools are sufficiently vetted and validated against measured data.

10.11.1 Modeling Tools

Modeling tools may provide insight on uncertainties identified in risk analyses that cannot be readily addressed by laboratory and/or field studies. Modeling population dynamics may be used to simulate the fate of the population or colony over years of exposure to the product, and/or at a wider scale than the field, and may have the potential to address generic questions such as colony-level implications from individual-level effects. Development of models for honey bees and non-*Apis* bees could thus address general questions such as:

- What level of mortality or brood loss is of minimal consequence at the colony or population level?
- What magnitude and frequency of effects on adult survival and brood success are required to put the viability of a honey bee colony at risk?
- How do these thresholds vary according to season?

Please provide in-text citation for the following references: "Alix & Lewis, 2011" "Alix et al., 2011" "Barrett et al., 1994" "Devillers et al., 2003" "Miles & Alix, 2011" "Vaughn et al., 2007" "Winston, 1987"

Answers to these generic issues are of great interest in conducting and interpreting risk assessments but also in support of decision making. The potential usefulness of modeling tools is discussed in more detail in Chapter 11.

REFERENCES

Abbott VA, Nadeau JL, Higo HA, Winston ML. 2008. Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 101:784–796.

Alix A, Lewis G. 2010. Guidance for the assessment of risks to bees from the use of plant protection products under the framework of Council Directive 91–414 and Regulation 1107–2009. *OEPP /EPPO Bull.* 40:196–203.

Alix A, Bakker F, Barrett K, Brühl CA, Coulson M, Hoy S, Jansen JP, Jepson P, Lewis, Neumann P, Süßenbach D, van Vliet P. 2011. ESCORT 3: Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals. Proceedings of the European Standard Characteristics of Non-Target Arthropod Regulatory Testing Workshop ESCORT 2, Zuiderduin, The Netherlands, March 21–23, 2000. March 8–11, 2010. In press.

Alston DG, Tepedino VJ, Bradley BA, Toler TR, Griswold TL, Messinger SM. 2007. Effects of the insecticide phosmet on solitary bee foraging and nesting in orchards of Captiol Reef National Park, Utah. *Environ. Entomol.* 36:811–816.

Barrett K, Grandy N, Harrisson EG, Hassan S, Oomen P. (eds). 1994. Guidance document on regulatory testing procedures for pesticides with non-target arthropods. *ESCORT Workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing)*. SETAC Publication, Wageningen, p. 53.

Please check and update the reference "Alix et al., 2011"

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Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet MC, Lewis G, Oomen PA, Schmuck R, Vogt H. 2001. Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. *ESCORT 2 Workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing)*. SETAC Publication, Wageningen, p. 46.

Devillers J, Decourtye A, Budzinski H, Pham-Delègue MH, Cluzeau S, Maurin G. 2003. Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. *SAR QSAR Environ. Res.* 14:389–403.

EC. 2010. Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (Directive 91/414/EEC).

European Food Safety Authority [EFSA]. 2009. Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. *EFSA J.* 7(12):1438. doi:10.2903/j.efsa.2009.1438

EPPO. 2010. Environmental risk assessment scheme for plant protection products. *OEPP /EPPO Bull.* 40:1–9.

Gels JA, Held DW, Potter DA. 2002. Hazards of insecticides to the bumble bees *Bombus impatiens* (Hymenoptera: Apidae) foraging on flowering white clover in turf. *J. Econ. Entomol.* 95:722–728.

George DA, Rincker CM. 1982. Residues of commercially used insecticides in the environment of *Megachile rotundata*. *J. Econ. Entomol.* 75:319–323.

Gradish AE, Scott-Dupree CD, Cutler GC. 2011a. Susceptibility of *Megachile rotundata* to insecticides used in wild blueberry production in Atlantic Canada. *J. Pest. Sci.* 85(1):133–140.

Gradish AE, Scott-Dupree CD, Frewin AJ, Cutler GC. 2011b. Lethal and sub-lethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*. *Can. Entomol.* 144:478–486.

Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G. 2010. Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest. Manag. Sci.* 66(2):142–146.

Hoerger F, Kenaga EE. 1972. Pesticide residues on plants: correlation of representative data as a basis for estimation of their magnitude in the environment. In: Coulston F, Korte F (eds), *Environmental Quality and Safety: Chemistry, Toxicology, and Technology.* Georg Thieme, Stuttgart, pp. 9–28.

Hodgson EW, Pitts-Singer TL, Barbour JD. 2011. Effects of the insect growth regulator, novaluron on immature alfalfa leafcutting bees, *Megachile rotundata*. *J. Insect Sci.* 11:43.

Huntzinger C, James RR, Bosch J, Kemp WP. 2008. Fungicide tests on adult alfalfa leafcutting bees *Megachile rotundata* (F.) (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 101:1088–1094.

Johansen CA, Rincker CM, George DA, Mayer DF, Kious CW. 1984. Effects of aldicarb and its biologically active metabolites on bees. *Environ. Entomol.* 13:1386–1398.

Konrad R, Ferry N, Gatehouse AM, Babendrier D. 2008. Potential effects of oilseed rape expressing oryzacystatin-1 (OC-1) and purified insecticidal proteins on larvae of the solitary bee *Osmia bicornis*. *PloS ONE.* 3(7):e2664. doi:10.1371/journal.pone.0002664

Ladurner E, Bosch J, Kemp WP, Maini S. 2005. Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie* 36:449–460.

Ladurner E, Bosch J, Kemp WP, Maini S. 2008. Foraging and nesting behavior of *Osmia lignaria* (Hymenoptera: Megachilidae) in the presence of fungicides: cage studies. *J. Econ. Entomol.* 101:647–653.

Macieira OJD, Hebling-Beraldo MJA. 1989. Laboratory toxicity of insecticides to workers of *Trigona spinipes* (F. 1793) (Hymenoptera: Apidae). *J. Apic. Res.* 28:3–6.

Mayer DF, Lunden JD. 1999. Field and laboratory tests of the effects of fipronil on adult female bees of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*. *J. Apic. Res.* 38:191–197.

Mayer DF, Kovacs G, Lunden JD 1998. Field and laboratory tests on the effects of cyhalothrin on adults of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*. *J. Apic. Res.* 37:33–37.

Michener CD. 2000. *The Bees of the World*. The John Hopkins University Press, Baltimore and London.

Miles MJ. Alix A. 2011. Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-*Apis* bees. In: Proceedings of the 11th ICPBR Meeting, Wageningen, The Netherlands, November 2011. Morandin LA, Winston ML, Franklin MT, Abbott VA. 2005. Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest. Manag. Sci.* 61:619–626.

Mineau P, Harding KM, Whiteside M, Fletcher MR, Garthwaite D, Knopper LD. 2008. Using reports of honey bee mortality in the field to calibrate laboratory derived pesticide risk indices. *Environ. Entomol.* 37(2):546–554.

Oomen PA, de Ruijter A, Van der Steen J. 1992. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *EPPO Bull.* 22:613–616.

Peach ML, Alston DG, Tepedino VJ. 1995. Sublethal effects of carbaryl bran bait on nesting performance, parental investment, and offspring size and sex ratio of the alfalfa leafcutting bee (Hymenoptera: Megachilidae). *Environ. Entomol.* 24:34–39.

Robinson GE. 1992. Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* 37:637–665.

Schmid-Hempel P, Heeb D. 1991. Worker mortality and colony development in bumblebees, *Bombus lucorum* (L.) (Hymenoptera, Apidae). *Bull. Soc. Entomol. Suisse.* 64:93–108.

Scott-Dupree CD, Conroy L, Harris CR. 2009. Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *J. Econ. Entomol.* 102:177–182.

Tasei JN. 2002. Impact of agrochemicals on non-*Apis* bees. In: Devillers J, Pham-Delegue M-H (eds), *Honey Bees: Estimating the Environmental Impact of Chemicals.* Taylor & Francis, New York.

Tasei JN, Carre S, Moscatelli B, Grondeau C. 1988. Recherche de la D.L. 50 de la deltamethrine (Decis) chez *Megachile rotundata* F. Abeille pollinistatrice de la luzerne (Medicago sativa L.) et des effets de doses infralethales sur les adultes et les larves. *Apidologie* 19(3):291–306.

Tasei JN, Ripault G, Rivault E. 2001. Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J. Econ. Entomol.* 94:623–627.

Thompson H. 2001. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie* 32:305–321.

Thompson HM, Hunt LV. 1999. Extrapolating from honeybees to bumblebees in pesticide risk assessment. *Ecotoxicology.* 8:147–166.

Torchio PF. 1983. The effects of field applications of naled and trichlorfon on the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius). *J. Kans. Entomol. Soc.* 56:62–68.

U.S. Environmental Protection Agency [USEPA]. 1992. Framework for Ecological Risk Assessment [EPA/630/R-92/001]. Risk Assessment Forum, Washington, DC.

U.S. Environmental Protection Agency [USEPA]. 1998. Guidelines for Ecological Risk Assessment [EPA/630/R-95/002F]. Risk Assessment Forum, Washington, DC.

U.S. Environmental Protection Agency [USEPA]. 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency Endangered and Threatened Species Effects Determinations. Office of Chemical Safety and Pollution Prevention, Washington, DC. http://www.epa.gov/espp/consultation/ecorisk-overview.pdf (accessed February 6, 2012).

U.S. Environmental Protection Agency. 2012a. Ecological Effects Test Guidelines. OCSSP 850.3030: Honey Bee Toxicity of Residues on Foliage [EPA 712-C-018]. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0154-0017.

Please provide the last accessed date for the website link in the reference "U.S. Environmental Protection Agency. 2012a".

U.S. Environmental Protection Agency. 2012b. User's guide for T-REX version 1.5 (Terrestrial Residue EXposure model). Environmental Fate and Effects Division, Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC. Available online at: http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm.

Valdovinos-Nunez GR, Quezada-Euan JJ, Ancona-Xiu P, Moo-Valle H, Carmona A, Sanchez ER. 2009. Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini). *J. Econ. Entomol.* 102:1737–1742.

Van der Steen JJM. 2001. Review of methods to determine the hazard and toxicity of pesticides to bumblebees. *Apidologie* 32:399–406.

Vaughn M, Shepherd M, Kremen C, Black SH. 2007. *Farming for Bees*. The Xerces Society, Portland, OR. p. 44.

Winston M. 1987. *The Biology of the Honey Bee*. Harvard University Press, Cambridge, MA. ISBN 0-674-07409-2.

Please provide last accessed date for the weblink in the reference "U.S. Environmental Protection Agency, 2012b."

Winston ML, Fergusson LA. 1985. The effect of worker loss on temporal caste structure in colonies of the honey bee (*Apis mellifera L.*). *Can. J. Zool.* 63:777–780.

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