Australian Government
Australian Pesticides and
Veterinary Medicines Authority

SEPTEMBER 2017

Roadmap for insect pollinator risk assessment in Australia

ISBN 978-1-925390-00-1 (electronic)

Ownership of intellectual property rights in this publication

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

Creative Commons licence

With the exception of the Coat of Arms and other elements specifically identified, this publication is licensed under a Creative Commons Attribution 3.0 Australia Licence. This is a standard form agreement that allows you to copy, distribute, transmit and adapt this publication provided that you attribute the work.

A summary of the licence terms is available from www.creativecommons.org/licenses/by/3.0/au/deed.en. The full licence terms are available from www.creativecommons.org/licenses/by/3.0/au/legalcode.

The APVMA's preference is that you attribute this publication (and any approved material sourced from it) using the following wording:

Source: Licensed from the Australian Pesticides and Veterinary Medicines Authority (APVMA) under a Creative Commons Attribution 3.0 Australia Licence.

In referencing this document the Australian Pesticides and Veterinary Medicines Authority should be cited as the author, publisher and copyright owner.

Use of the Coat of Arms

The terms under which the Coat of Arms can be used are set out on the Department of the Prime Minister and Cabinet website (see www.dpmc.gov.au/pmc/publication/commonwealth-coat-arms-information-and-guidelines).

Disclaimer

The material in or linking from this report may contain the views or recommendations of third parties. Third party material does not necessarily reflect the views of the APVMA, or indicate a commitment to a particular course of action.
There may be links in this document that will transfer you to external websites. The APVMA does not have responsibility for these websites, nor does linking to or from this document constitute any form of endorsement.
The APVMA is not responsible for any errors, omissions or matters of interpretation in any third-party information contained within this document.

Comments and enquiries regarding copyright:

Director Public Affairs and Communication
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604 Australia

Telephone: +61 2 6210 4701

Email: communications@apvma.gov.au

This publication is available from the APVMA website: www.apvma.gov.au.

CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

An APVMA regulatory workshop on 'Pesticides and the Health of Insect Pollinators', held on 24 July 2013, discussed several risk assessment frameworks available for insect pollinators and decided that the framework described in US EPA et al (2012) was the most suitable for adoption in Australia. This framework appears more specific to honey bees and, as noted at the workshop, commercial pollination services in Australia are essentially all provided by *Apis mellifera*. Nevertheless, the APVMA and its primary environmental advisory agency, the Department of the Environment, considered that there are elements of European guidance documents which could be combined with the North American approach to develop a risk assessment methodology to suit Australia's agricultural and regulatory environment.

This 'roadmap' is a short guide to conducting risk assessments for bees and other insect pollinators. It will be used by the APVMA to guide its risk assessments of proposed uses of pesticide products which may impact on these organisms. It draws from international approaches described in various source documents from Europe and North America.

The framework that has been adopted allows for a tiered approach to risk assessment. The first tier of assessment involves the traditional assessment approach of calculating risk quotients, and methods for refinement at this tier are described, relying on data and approaches from both Europe and North America.

At higher tiers of assessment, increasingly complex studies pertaining to exposure and effects (semi-field and full-field studies at colony level) are considered. These studies allow for refinements in exposure and/or effects estimations using an increasing level of realism. Importantly, and as noted in the North American guidance document, the different levels of refinement are not intended to be prescriptive. The specific set of data used in assessing potential risks of a pesticide to bees ultimately depends on multiple lines of evidence and risk management objectives.

As the science in this field continues to evolve, additional data requirements and assessment methodology may be incorporated into the risk assessment.

1 INTRODUCTION

The APVMA held a regulatory workshop ('Pesticides and the Health of Insect Pollinators') on 24 July 2013 to address the following issues with respect to assessing the risks of pesticide use to honey bees and other insect pollinators:

1. Consideration of the suite of tests currently available and those being developed to examine:
   a) the effects of pesticides on bees and other insect pollinators
   b) the potential extent of exposure of bees and other insect pollinators to pesticides
2. The establishment of appropriate protection goals for insect pollinators and the linking of data requirements and risk assessment methodology to these protection goals.
3. The development of a more consistent approach to pollinator protection statements on labels of pesticide products.

Workshop participants agreed on a number of recommendations relating to data requirements, pollinator protection goals, a suitable pollinator risk assessment framework for Australia, and the development of label statements relating to pollinator protection.

With regard to a risk-assessment framework, workshop participants decided that the framework described in a North American White Paper[1] (US EPA et al, 2012) was the most suitable for adoption in Australia. This framework appears more specific to honey bees and, as noted at the workshop, commercial pollination services in Australia are essentially all provided by *Apis mellifera*. Nevertheless, the APVMA and its primary environmental advisory agency, the Department of the Environment, considered that there were elements of European pollinator risk-assessment guidance which could be combined with the North American approach to develop a risk assessment methodology to suit Australia's agricultural and regulatory environment.

The 2012 North American White Paper gave rise to a pollinator risk assessment guidance document ('Guidance for Assessing Pesticide Risks to Bees') which was published on 19 June 2014 by the US Environmental Protection Agency, the Canadian Pest Management Regulatory Agency and the California Department of Pesticide Regulation[2]. It describes a tiered process for evaluating potential adverse effects on pollinating bees from exposure to pesticides. If exposure is considered likely, then modelled exposure estimates or conservative default values are compared against laboratory-based toxicity estimates for individual bees. Empirical data on residues in pollen and nectar may be used to refine exposure estimates. The risk assessor calculates risk quotients (that is, measures of exposure divided by the relevant toxicity endpoint), which are then compared to a level of concern (Tier-1). Information from open literature, unpublished registrant studies, and bee kill incidents may further characterise potential risks. Tier-2 incorporates toxicity studies conducted at the colony level, either within a tunnel enclosure or in an open field using colonies provided with spiked diets at known concentrations. If further refinement is necessary, Tier-3 field studies are conducted under real-world exposure scenarios to address specific uncertainties identified in lower-tier studies. Tiers 2 and 3 toxicity studies, in combination with residue studies of pollen and nectar, may further characterise potential risk(s). Exposure and toxicity data focus on the

[1] www.cdpr.ca.gov/docs/emon/surfwtr/presentations/epa_whitepaper.pdf

[2] www2.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

honey bee (*Apis mellifera*) as a surrogate for all pollinating bees, but when available, data on other non-*Apis* bees may be included in the risk assessment. The overall process for assessing potential risks to bees depends on multiple lines of evidence, and risk management options may be considered at any tier-1n the process.

A very detailed European Food Safety Authority (EFSA) guidance on conducting assessments of the risks to bees from the use of plant protection products (PPPs) and their active constituents was published in July 2013 (updated on 4 July 2014[3]).

This guidance document or 'roadmap' for pollinator risk assessment references the above two documents. It will be used by the APVMA to guide its risk assessments of proposed uses of pesticide products which may impact on these organisms. The document will be reviewed periodically to reflect ongoing advances in testing and assessment methodologies.

1.1 Source documents

It is not the intention of this document to repeat in detail the methods and guidance provided in the source documents; this report should be read in conjunction with these documents, identified as follows:

- US EPA et al (2014): Guidance for Assessing Pesticide Risks to Bees
- US EPA et al (2012): White Paper in Support of the Proposed Risk Assessment Process for Bees
- EFSA (2013): Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees).

The following guidance document regarding exposure and effects assessment needs to be considered in the context of the overall frameworks for foliarly-applied chemicals (Appendix A) and seed treatment/soil-applied chemicals (Appendix B).

[3] www.efsa.europa.eu/en/efsajournal/pub/3295.htm

2 AUSTRALIA'S PROTECTION GOALS

The protection goals around which the risk assessment for bees and other insect pollinators is conducted in Australia, was considered at the APVMA regulatory workshop on 'Pesticides and the Health of Insect Pollinators' on 24 July 2013. Essentially, the protection goals are separated between commercial activities and ecological targets. The following table summarises these goals and indicates the assessment end-points (US EPA et al, 2014) and measurement end-points (US EPA et al, 2014) that may be used to inform the risk assessment.

Table 1: Protection goals and examples of associated assessment and measurement (population and individual) end-points for bees and other insect pollinators

		MEASUREMENT END-POINTS	
PROTECTION GOALS	ASSESSMENT END-POINTS	POPULATION LEVEL AND HIGHER	INDIVIDUAL LEVEL
COMMERCIALLY-ORIENTATED PROTECTION GOALS			
Protection of pollination services	Population size and stability of native bees and commercially managed bees	Colony strength and survival Colony development	Individual worker and larval survival assays; queen fecundity; brood success; worker bee longevity
Protection of honey production and other hive products provided by honey bees (*Apis mellifera)*[1]	Quantity and quality of hive products	Quantity and quality of hive products including pesticide residue levels on honey/wax	Individual worker and larval survival assays; queen fecundity/ reproduction; larval emergence
ECOLOGICALLY-ORIENTATED PROTECTION GOAL			
Protection of pollinator biodiversity[2]	Species richness and abundance[3]	Individual bee survival (solitary bees) and colony strength and survival (social bees) Species richness and abundance	Individual worker and larval survival assays; queen fecundity/ reproduction; larval emergence

(1) Commercial pollination services in Australia are essentially all provided by Apis mellifera, with insignificant contributions from non-Apis bees and native bees. (2) Protection of adequate numbers and kinds of bee species that contribute to the health of the environment (primarily non-Apis bees). (3) Use of honey bees as a surrogate for other insect pollinators has limitations; however, it is assumed that, as with all surrogates, data on individual organisms as well as colony-level data would provide some relevant information on the potential effects of a pesticide on both solitary bees as well as 'eusocial' taxa. In addition, protection of honey bees would help contribute indirectly to pollinator diversity by assisting in the propagation of the many plants species pollinated by honey bees, plants which also serve as food sources for other pollinating native insects.

3 DATA REQUIREMENTS

There has been significant discussion internationally relating to data necessary to adequately assess the exposure and ecotoxicity of pesticides to bees and other pollinators. Currently there is a lack of harmonised international test guidelines for testing of pesticides on bees and other insect pollinators. However, there has been, and there is ongoing consideration at an international level, of a common suite of test data requirements to support the assessment of both exposure and toxicity.

The 2013 APVMA regulatory workshop on 'Pesticides and the Health of Insect Pollinators' considered the suite of tests currently available, and those being developed, that will allow assessment of the potential extent of exposure of bees and other insect pollinators to pesticides and the effects of pesticides on bees and other insect pollinators.

It was recognised by the workshop that the APVMA data guidelines for testing of insecticides do not appear adequate to properly consider possible routes and extent of exposure of insect pollinators to pesticides or to assess the potential for adverse effects (including sub-lethal effects) of pesticides on honey bees and other insect pollinators.

3.1 Current Australian data guidelines

The current Australian data guidelines for effects of pesticides on bees are described in the 'Environmental Risk Assessment Guidance Manual for Agricultural and Veterinary Chemicals' (SCEW, 2009), available at www.scew.gov.au/resource/chemical-risk-assessment-guidance-manuals.

3.2 International activities regarding data requirements

Over the past several years the testing requirements on effects and exposure to bees and other insect pollinators have been discussed extensively internationally. Activities in this field are described below.

Some regional declines in native, feral and managed pollinator populations have resulted in significant concerns and increased global dialogue about the potential factors that may be causing these declines. In an effort to further this dialogue, the Society of Environmental Toxicology and Chemistry (SETAC) held a Pellston Workshop in January 2011 to explore the state-of-the-science on pesticide risk assessment for pollinators (Fischer & Moriarty, 2011). The workshop built upon the extensive efforts of different organisations, regulatory authorities, and individuals, both nationally and internationally, with the aim of achieving a better understanding of the effects of pesticides on the health and behaviour of native and honey bees. Australia was represented at the workshop.

This Pellston Workshop[4] focused on four key areas:

1. Design/identify testing protocols to provide an understanding of potential exposure of bees, to pesticide residues in pollen and nectar (including daily ingestion rates), as well as exposure through other routes of direct and indirect exposure.

[4] SETAC workshops are known as Pellston Workshops in recognition of the inaugural 1977 global workshop that brought leading scientists from academia, business, and government to Pellson, MI, USA

2. Design/identify testing protocols to measure effects of pesticides to developing brood and adult honey bees at both the individual and colony level.
3. Propose a tiered approach for characterising the potential risk of pesticides to pollinators.
4. Explore the applicability of testing protocols used for honey bees (*Apis*) to measure the risk of pesticides to native (non-*Apis*) insect pollinators.

In 2010, the OECD Working Group on Pesticides (WGP) reviewed a proposal for a project related to pollinator declines and agreed to four activities, one of which was pollinator testing requirements. Pollinator testing requirements are being considered by the Pesticide Effects on Insect Pollinators (PEIP) Project which was proposed to occur in phases. In Phase 1, work group members were (amongst other activities) to collect and share information regarding current exposure and effect testing methods and how the results from these studies are used to inform the risk assessment process. Australia is represented on this group.

In addition to the US EPA White Paper (US EPA et al, 2012), the European Food Safety Authority (EFSA) Scientific Opinion (EFSA, 2012) and the SETAC Global Pellston Workshop (Fischer & Moriarty, 2011), testing requirements relating to the determination of exposure to pesticides and their effects on pollinators have been extensively discussed in other expert working groups including the International Commission for Plant-Pollinator Relationships (ICPPR) and the European Plant Protection Organization (EPPO). The conclusions and recommendations of the ICPPR and EPPO working groups have been published and, in the case of the EPPO, were adopted by participating EU Member States in October 2011 (onlinelibrary.wiley.com/doi/10.1111/j.1365-2338.2010.02419.x/pdf).

Members of the OECD PEIP used documentation from these sources to list the available testing guidelines. In addition, the group identified additional work that needed to be completed, but this is as yet incomplete and outside the scope of this current document.

The following tables (Table 2 and 3) describe the currently-available test guidelines or exposure defaults (either available or being developed) for consideration of exposure of pollinators to pesticides. Current Australian data guidelines in the 'Environmental Risk Assessment Guidance Manual for Agricultural and Veterinary Chemicals' (SCEW, 2009) have been highlighted in Table 2.

3.3 Exposure data for bees

As noted in Fischer & Moriarty (2011), it is important that exposure routes that 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 to be able to assess provided toxicity data. To date, these types of data have not been commonly available in Australia.

Table 2: Summary of available methods examining the potential EXPOSURE of bees to pesticides (adapted from OECD PEIP information)

AREA/TYPE OF TEST	BACKGROUND INFORMATION
Estimation of level of residues in pollen and nectar resulting from spray treatment of crops	Default values have been proposed in the Pellston proceedings (2011), the US EPA white paper (US EPA et al, 2012) and the EFSA guidance document (EFSA, 2013). These values reflect a limited database
Estimation of level of residues in pollen and nectar resulting from soil/seed treatment	The current default value of 1 mg/kg was derived from a database of residue concentrations in whole plants (EPPO, 2010a). It overestimates the residue concentrations in pollen/ nectar This screening value was adopted to represent a Tier-1 exposure value for soil applications, based on the maximum value from data compiled by Alix et al (2009), including pesticide residues measured in different plant parts (leaves, fruit, green part, inflorescence, whole plant and grain) following applications to soil or seed treatments (US EPA et al, 2012)
Estimation of level of residues in pollen/nectar by calculation based on the spray application rate	US EPA white paper (US EPA et al, 2012) and EFSA guidance document (EFSA, 2013)
Estimation of guttation fluid[5] residue levels and potential relevance of exposure resulting from soil application of granules and seed treatment	Exposure route considered as minor, to be considered on a case-by-case basis in the US EPA white paper and in the EFSA guidance document
Exposure from dusts generate at sowing of coated seeds	Results from studies from the German Julius Kühn Institut (JKI) and other sources of data indicate potential for exposure for certain seeds, drillers, weather conditions and coinciding flowering (see USEPA et al, 2012 and EFSA, 2013)

The exposure group in the SETAC Global Pellston Workshop (Fischer & Moriarty, 2011) identified higher-tier studies to refine the exposure assessment such as a Tier-2 contact toxicity study and Tier-3 semi-field (tunnel[6]) tests to refine the oral exposure assessment.

3.4 Effects (hazard) data

The 2013 APVMA regulatory workshop 'Pesticides and the Health of Insect Pollinators' recommended the following with respect to toxicity data:

Commercial pollination services in Australia are essentially all provided by *Apis mellifera*, with contributions from non-*Apis* bees and native bees currently being insignificant. This is an important point that has implications for

[5] 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 et al, 2009).

[6] The benefit of these studies is that they facilitate the study of whole colonies that can be confined in tent enclosures (tunnels) for limited periods of time (US EPA, 2011).

protection goals and the risk assessment framework. It also has implications for data requirements. The following recommendations pertaining to suitable data to allow an assessment of risk were made by the workshop:

1. Retain the need for adult acute contact and oral tests.
2. Require a 10-day adult and larvae study when exposure is possible OR if there is a reason for concern about the toxicity of the active constituent/formulation being assessed.
3. The need for higher-tier (*Apis*) studies will be assessed on a case-by-case basis.
4. Non-*Apis* and Australian native bees are not considered likely to have significant in-crop exposure for broad-acre cropping in the context of provision of pollination services. At this stage no separate tests are required for non-*Apis* bees and other insect pollinators.
5. Where persistence and/or residual toxicity is a problem, at a minimum, the need for a study on the toxicity of residues on foliage remains a requirement.

Some important considerations with respect to honey bee toxicity testing were discussed at the 2011 SETAC workshop (Fischer & Moriarty, 2011) and are summarised as follows:

> Currently, no globally-harmonised tiered testing system for honey bees exists. Participants at the SETAC Global Pellston Workshop pointed out the benefits of harmonising the EU and US systems. The Organisation for Economic Cooperation and Development's (OECD's) oral and contact tests for the assessment of the acute toxicity of a compound to adult bees could be easily adopted and offer distinct advantages for improving risk assessment in the USA and other countries.
>
> Currently there are no standardised required tests to address chronic toxicity to adult bees or larvae, with exposure either by contact or by ingestion. Studies have shown that it is possible to conduct an adult bee chronic toxicity test for 10 to 14 days to calculate the NOAEC, but a standardised feeding protocol needs to be developed to ensure consistency/repeatability across these studies.
>
> The risk to bee brood[7] has been investigated in the past only if the active ingredient was an insect growth regulator (IGR). The Pellston Workshop recommended that a Tier-1 chronic oral test with larvae be adopted for all compounds likely to be encountered by honey bee brood. Analogous to Tier-1 adult bee testing, consideration should be given to testing not only active ingredients, but end-product formulations if two or more active ingredients in formulated products are expected to exhibit greater than additive toxicity.

Progress in relation to the development of some of the tests on bee brood is described in the text following Table 3 below.

[7] The term 'bee brood' refers to the eggs, larvae and pupae.

Table 3: Summary of available tests examining the potential EFFECTS of pesticides to bees (adapted from OECD PEIP information). Highlighted cells indicate current Australian data requirements

AREA/TYPE OF TEST	GUIDELINE
Acute oral test for adult honey bees	OECD, 1998a (Test Guideline 213)
Acute contact test for adult honey bees	OECD, 1998b (Test Guideline 214) US EPA, 1996a. (OPPTS 850.3020)
Honey Bee (*Apis mellifera* L.), Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory	OECD Test Guideline has been developed but is in draft form (as at the date of this document). Based on published method: Decourtye et al, 2005;
Toxicity of residues on foliage	US EPA, 2012a. (OPPTS 850.3030)
Honeybee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure.	An OECD guidance document has been developed but is yet to be published (as at the date of this document). Based on published methods: Aupinel, 2005.
Semi-field test on larval development of honey bees (micro colonies)	Oomen et al, 1992 Schur et al, 2003 OECD, 2007
Test on the impact of contaminated pollen/nectar on honey bees prior to high tier testing	This test could be adapted from the Oomen (1992) feeding study; however, the focus is now on the entire colony as opposed to on the larvae alone
Tunnel test on honey bees (semi-field test)	OECD, 2007
Field test on honey bees	EPPO, 2010 US EPA, 1996b (OPPTS 850.3040)

Honey bee (*Apis mellifera*) larval toxicity test—single exposure. There is now a published OECD test guideline describing a honey bee brood acute toxicity test under laboratory conditions. The test (OECD Test Guideline 237) was published on 26 July 2013 and tests the toxicity of a substance on larvae fed with spiked food as a Tier-1 test. The method aims to determine the LD50 at 72 h following a single exposure (OECD, 2013).

In addition, an OECD guidance document on a honey bee larval toxicity test following repeated dose exposure was approved in April 2016; this **Honey Bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure** document describes a honey bee brood acute toxicity test under laboratory conditions using repeated doses. The method aims to determine the No Observed Adverse Effect Concentration (NOAEC) on larvae, pupae and adults following a repeated exposure of larvae to a substance. Essentially, on day 1 of the study, first instar synchronised larvae (larvae of the same age) are taken from the comb of three colonies and individually placed into 48 well-plates where they are fed a standardised amount of artificial diet. From day 3 until day 6 of the test, the test chemical is administered daily to the larvae with the diet in a range of five increasing concentrations. Mortalities are recorded daily from day 4 to day 7 of the test. The NOAEC can be determined at day 7 on larvae, at day 15 on pupae and at day 22 on adults.

Current Australian data requirements (highlighted in Table 2) are those identified in the 'Environmental Risk Assessment Guidance Manual for Agricultural and Veterinary Chemicals' (SCEW, 2009). The acute adult contact and oral toxicity tests are part of the standard data requirements. However, the other two types of tests identified as current Australian data requirements (*viz*. residues on foliage and semi-field tests) are not always needed.

Toxicity of residues on foliage is a US EPA test guideline where toxicity testing of foliar residues is typically conducted for 24 h then expanded at 24 h intervals depending on the results. Limitations of the test are that endpoints are limited to mortality to young adult worker bees and clinical signs of intoxication (sub-lethal effects) are not well quantified (US EPA, 2011).

The fourth possible Australian data requirement is a bee-brood feeding study. The requirement is identified in Table 3 (above) as 'Tunnel test on honey bees', and some guidance is found in OECD Guidance Document 75 (OECD, 2007). That document provides a sequential testing strategy for determining effects on bee brood, and the tunnel test component is based on several studies and EPPO guideline No. 170. Essentially, small healthy honey bee colonies are placed in tunnel tents shortly before full flowering of the crop. The time period in the tunnels includes approximately 2–3 days before the treatment to acclimatise and a further 7 days after application for direct exposure. Following exposure in tents the colonies are placed in areas where no attractive main crops are available, ideally within a radius of 3 km to ensure that the contaminated food in the test colonies will be assimilated by the colony. Mortality of honey bees, flight activity, and condition of the colonies and development of the bee brood are evaluated several times over a period of at least 4 weeks after the initial brood assessment. Results are evaluated by comparing the treated colonies with control colonies (exposed to water-treated crop) and with colonies exposed to a positive control reference chemical (OECD, 2007).

The requirement for these tests is considered on a case-by-case basis and depends on factors such as the toxicity and proposed use pattern of the chemical under consideration. Examples for which such data (tunnel tests/field studies) may be required, regardless of the available Tier-1 toxicity tests, include for insect growth regulators, toxicants with delayed action that may be transported back to hives, and neonicotinoids where there may be effects on growth or development of bees.

Tiered approach to effects assessment

The following tables are described in US EPA et al, 2014 and explain the expected end-points and consideration of the strengths and limitations of various bee toxicity studies.

Table 4: Tier-1 – End-points, strengths and limitations of bee toxicity studies

STUDY TYPE	PRIMARY END-POINTS	STRENGTHS	LIMITATIONS
Adult, Acute contact	Mortality, contact LD50	• Quantifiable test doses • Dose-response curve is generated • Some sub-lethal effects can be measured	• Only 1 exposure route • NOAECs not typically generated • Acute exposure only • Sub-lethal effects measurement limited in scope • Effects are assessed at the individual level
Adult, Acute oral	Mortality, oral LD50		

STUDY TYPE	PRIMARY END-POINTS	STRENGTHS	LIMITATIONS
Adult, Chronic oral	Mortality, NOAEC	• Quantifiable test doses • NOAEC and/or dose-response curve is generated • Some sub-lethal effects can be measured	• Only 1 exposure route • Sub-lethal measurement limited in scope • Effects are assessed at the individual level • Test is currently under development
Larval acute (single dose)	Mortality, larval LD50/NOAEC	• Quantifiable test doses • NOAEC and/or dose-response curve is generated • Contact and oral exposure routes are included • Larval effects are important for some MOAs (insect growth regulators)	• Actual consumed dose may vary • Assessment of effects through pupation is currently difficult • Effects are assessed at the individual level • Test is currently under development (chronic)
Larval chronic (repeat dose)	Adult emergence/NOAEC		
Foliar residue	Mortality, residual toxicity and/or RT25	• Contact exposure through residues on foliage • Can assess pesticide residual toxicity	• Acute exposure only • Actual dose not quantified • Effects are measured at the individual level

Table 5: Tier-2 – End-points, strengths and limitations of bee toxicity studies

STUDY TYPE	PRIMARY END-POINTS	STRENGTHS	LIMITATIONS
Semi field, Tunnel	Colony strength Brood pattern and development Foraging activity Worker mortality and behaviour Food storage and consumption Queen health	• Multiple exposure routes (contact, oral) related to pesticide use methods • Minimises influence of outside exposure to other chemicals • Some behavioural end-points can be quantified; • Standard test protocol (OECD) • Colony level effects can be related to application rate and/or residues	• Short term exposure only (usually 7–10 days in the tunnel) • Foraging may not be natural; • Stress on colonies from tunnel confinement • Replication and statistical power are often low • Usually based on a surrogate crop

STUDY TYPE	PRIMARY END-POINTS	STRENGTHS	LIMITATIONS
Semi field, Feeding		• Long term exposure can be assessed • Colony level effects can be related to dietary concentration • Greater replication can be achieved vs. tunnel or full field studies • Greater control over exposure vs. full field studies	• Oral route only • Consumed dose may differ from that encountered in the field • Protocols have not been standardised • Confounding influences of off-site foraging and exposure • Foraging may not be natural

Table 6: Tier-3 – End-points, strengths and limitations of bee toxicity studies

STUDY TYPE	PRIMARY END-POINTS	STRENGTHS	LIMITATIONS
Full field (experimental)	Colony strength Brood pattern and development Foraging activity Worker mortality and behaviour Food storage and consumption Queen health	• Most environmentally realistic of crop/pesticide exposure conditions • Can resolve specific uncertainties raised from lower tiers	• Practical constraints may limit ability to assess 'high end' exposure scenarios • Replication and statistical power often low • Confounding influences of off-site foraging and exposure • Costly
Full field (monitoring)	Colony strength Brood pattern and development Worker mortality and behaviour Food storage and consumption Queen health	• Most environmentally realistic of crop/pesticide exposure conditions • Can incorporate multiple crop exposure scenarios	• Exposure may be difficult to interpret • Causal linkages may be confounded by other stressors • Costly

3.5 Non-*Apis* bees and other insect pollinators

As discussed in Fischer & Moriarty (2011), 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 a surrogate for the many non-*Apis* species. The development of standardised contact and oral toxicity tests for non-*Apis* species (adults and larvae) has yet to be completed and ring tested[8], but is seen as a highly desirable focus area for advancing the ecological relevance of the tiered testing system and in reducing uncertainty in bee toxicity testing. Based on unpublished data on the effects of various pesticides on 21 tested non-*Apis* species, it appears that

[8] A multi-laboratory validation study in which all laboratories test the same substances using identical test protocols. The purpose of the study is to determine inter- and intra-laboratory reproducibility of a test method.

LD50 values for several species are within an order of magnitude of the value for the honey bee (*ibid*). Limited data for pesticides with newer chemistries suggest wider variations in the toxic levels (LD50) between *Apis* and non-*Apis* species. Thus, further data are required to confirm whether or not the toxicity for adult non-*Apis* bees can be predicted from that for *Apis mellifera* adults. 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.

At the 2013 APVMA regulatory workshop on 'Pesticides and the Health of Insect Pollinators', there was a recommendation not to require separate tests for non-*Apis* bees and other insect pollinators. Therefore, with the exception of *Apis* toxicity data, and standard non-target arthropod data, no specific information for non-*Apis* and other insect pollinators is likely to be received.

4 RISK ASSESSMENT—HONEY BEES, TIER-1

The following methodology should be considered in conjunction with the framework shown at Appendix A (foliar application) and Appendix B (seed treatment/soil applied).

The Tier-1 assessment results in calculation of risk quotients (RQs). While the 2013 APVMA regulatory workshop agreed with the risk assessment framework described in US EPA et al (2012), there was no discussion on the level of concern (LOC) applied to the risk quotients. Currently, Australia uses a standard LOC of 1.0 for honey bee risk assessment using acute adult oral and contact toxicity test data (SCEW, 2009). However, in US EPA et al (2012) the LOC to which the acute RQ value is compared is set to 0.4, based on the historic average dose-response relationship for acute toxicity studies with bees and a 10 per cent mortality level.

4.1 Exposure routes

The first step of the risk assessment is to determine the potential for exposure to adult bees and bee brood (stages 2a and/or 2b of the frameworks).

Foliar spray applications

In general, adult bees and their brood (eggs, larvae, pupae) are exposed to pesticides if they are applied to pollinator-attractive crops or drift to pollinator attractive plants during periods when bees are likely to be foraging. Exposure to bee brood and other castes of bees in hives is expected when exposure to foraging bees is identified, as foragers will bring residues back to the hive. Pre-bloom foliar application of pesticides to pollinator attractive crops may also result in exposure to bees if the pesticide is persistent and translocates to pollen and nectar after spray application (US EPA et al, 2014). Appendix D in EFSA (2013) provides useful information regarding the attractiveness of main agricultural crops to bees, which can be considered in the exposure assessment.

The following major exposure routes for foliar spray applications as per the conceptual model outlined in Appendix A1.1 and Appendix A1.2 provided in the North American ‘Guidance for Assessing Pesticide Risks to Bees’ (US EPA et al, 2014).

Table 7: Major exposure routes and receptors—foliar spray application

SOURCE	ROUTE	RECEPTORS
Spray deposition on bees	Contact	Foraging bees
Deposition on plants Residues on plant surfaces Residues on pollen and nectar	 Contact Oral (ingestion)	 Foraging bees Foraging bees Hive bees (pollen and nectar processing; comb production) Bee brood (brood food) Queen (royal jelly)

Other exposure routes include deposition onto soil and surface water. Resulting residues in soil and surface water are not considered to be major exposure routes in the case of non-systemic substances. However, for systemic substances, a further exposure route for residues in pollen, nectar, exudates and honey dew may become relevant, based on foliar translocation of residues on plant surfaces, or from root uptake of residues in the soil.

Exposure to residues in drinking water

While exposure to residues in water are not considered to be a major exposure route, EFSA (2013) does consider assessment of risk from exposure to contaminated water (see Section 4.2, p 54 of the EFSA bee risk assessment document).

Seed treatment applications

The following major exposure routes for systemic seed-treatment chemicals (which are absorbed and act systemically) is based on the conceptual model (Appendix A1.3) provided in US EPA et al (2014):

Table 8: Major exposure routes and receptors—seed treatment

SOURCE	ROUTE	RECEPTORS
Drift of abraded seed coatings	Contact	Foraging bees
Residues on planted seed Residues in pollen, nectar, guttation fluid, honey dew	Oral (ingestion)	Foraging bees Hive bees (pollen and nectar processing; comb production) Bee brood (brood food) Queen (royal jelly)

Dust exposure

Deposition of dust from abraded seed coatings onto soil, plants and surface water may constitute a significant route of exposure.

Currently, methods of consistently quantifying exposure to dusts have not been well defined. The extent to which honey bees are exposed *via* contact with abraded seed coat dust is determined by many factors, including the physico-chemical properties of the seed coating, seed planting equipment, use of seed delivery agents (eg, talc and graphite), environmental conditions (wind speed, humidity), and hive location in relation to sowing. These factors influence the amount of dust formed, the pesticide concentration in the dust, and depending on dust particle size and weather, the extent to which the dust may move (US EPA et al, 2012). Appendix C of the EFSA bee risk assessment guidance (EFSA, 2013) is dedicated to the relevance of dust from treated seeds.

Due to a lack of harmonised methodology in assessing risk to bees from dust exposure, the APVMA does not currently require a quantitative assessment of risk from this exposure route. However, if exposure through dust from abraded seed coatings is possible based on use pattern, the issue should be considered qualitatively, taking into account management options to limit exposure.

In August 2014, CropLife Australia released their Seed Treatment Stewardship Strategy. This Strategy is part of CropLife Australia's Pollinator Protection Initiative and includes four specific stewardship and best practice guides; these outline measures to reduce risks from dust generated during handling and planting of treated seed and provides guidance on industry best practices to minimise off-target movement of pest and disease management products. The Strategy complements existing strategies for stewardship of treated seed, like those developed by the Australian Seed Federation (ASF) and the Rural Industries Research and Development Corporation (RIRDC).

Two specific components of the strategy pertinent to this assessment are the 'Treated seed planting stewardship guide' and the 'Best management practice guide for planting insecticide treated seed'. The strategy is available at: www.croplife.org.au/wp-content/uploads/2014/08/CropLife-Seed-Treatment-Stewardship-Strategy-LOW-RES.pdf. This document contains some information regarding practices to avoid dust generation during handling and planting.

As methodology and/or data relating to bees risk assessment from this exposure route evolve, the requirement for a quantitative assessment may change.

Guttation fluid

EFSA (2013) discusses the assessment of risk from exposure to contaminated water with the assessment risk from exposure to guttation water specifically at Section 4.1 (p 28) of that document. This sub-section discusses uncertainties and methodology pertaining to exposure estimation, including refinement options along with risk mitigation options.

It is noted in Table 2 that guttation fluid is considered to be only a minor route of exposure. The risk from exposure to neonicotinoids in guttation fluid is considered to be low[9].

Given this, the APVMA does not currently require a risk assessment to bees based on exposure to guttation fluid. This position may be changed in the future should information be provided that indicates a need for risk assessment through this exposure route.

Soil applied substances

The following major exposure routes for systemic soil applied substances is based on the conceptual model (Appendix A1.4) provided in US EPA et al (2014).

Residues in surface water resulting from runoff or erosion is not considered a major exposure route for bees.

[9] No incidents of field mortalities of bees from exposure to neonicotinoids in guttation fluid of oilseed rape had been reported in Europe during 15 years of monitoring (Pistorius, 2014).

Table 9: Major exposure routes and receptors—soil applied

SOURCE	ROUTE	RECEPTORS
Residues in soil Residues on pollen, nectar, exudates, honey dew (root uptake)	Oral (ingestion)	Foraging bees Hive bees (pollen and nectar processing; comb production) Bee brood (brood food) Queen (royal jelly)

4.2 Foliar spray

Adult, contact exposure

Step 1 (Box 3a of Framework at Appendix A) – Calculate Tier-1 PEC for **Adult contact exposure**.

Australian methodology: Previously, Australia determined the risk to bees based on the assumption of their surface area being 1 cm^2, and directly comparing the application rate to the contact LD50. The exposure calculation was therefore:

$$PEC_{Contact} = (AR\ X\ 10)\ \mu g\ ac/bee$$

Where: AR = application rate (kg ac/ha)

Updated methodology (US EPA et al, 2014): The exposure estimate for adults (individual survival) through contact is provided in the following equation:

$$PEC_{Contact} = AR\ X\ 2.4\ \mu g\ ac/bee$$

Where: AR = application rate (kg ac/ha).

The rationale behind the value of 2.4 µg/bee is explained in the White Paper (US EPA et al, 2012). Essentially, it is the maximum concentration (normalised to an application rate of 1 kg ac/ha) from studies undertaken wherein which bees were foraging in apple orchards and *phacelia*, such that they could have been directly sprayed, or come in contact with treated foliage.

Step 2 (Box 4a of Framework) – Calculate Tier-1 Risk Quotients (RQs) for **Adult contact exposure**.

The $PEC_{Contact}$ is compared to the adult contact LD50 (µg/bee) to generate the risk quotient:

$$RQ = PEC_{Contact}/Acute\ LD50_{Contact}$$

The RQ is compared to the level of concern (LOC) for interpretation. The framework described here applies a LOC for acute exposure of 0.4.

Where risk quotients exceed the level of concern, further refinement is required – see Section 4.5 below for initial exposure refinement options.

Adult, oral exposure

Step 1 (Box 3b of Framework at Appendix A) – Calculate Tier-1 PEC for Adult oral exposure.

The exposure estimate for adults (individual survival) through diet is provided in the following equation:

$$PEC_{Adult,Oral} = AR\ X\ \left(98\frac{\mu g\ ac}{g}\right) X\ 0.292\frac{g}{day}$$

Where: AR = application rate (kg ac/ha).

The rationale behind the value of 98 µg/g residues is explained in the US EPA White Paper (US EPA et al, 2012). Essentially, it equates to the upper-bound (90th percentile) residue for tall grass normalised to an application rate of 1 kg ac/ha and is considered to represent an upper-bound residue value for pollen and nectar. This value is then converted to a dietary dose received by adult worker bees using a pollen and nectar consumption rate of 292 mg/day (median value obtained for nectar foragers).

Step 2 (Box 4b of Framework) – Calculate Tier-1 Risk Quotients (RQs) for **Adult oral exposure**.

For the acute assessment, the $PEC_{Adult,\ oral}$ is compared to the adult contact LD50 (µg/bee) to generate the risk quotient:

$$RQ_{acute} = PEC_{Adult,oral}/Acute\ LD50_{Adult,oral}$$

The RQ is compared to the level of concern (LOC) for interpretation. Australia uses a standard LOC of 1.0 for the honey bee risk assessment, and revision of this value has yet to be discussed. The US EPA et al (2014) states indicates use of a LOC for acute exposure of 0.4. EFSA (2013) accepts a hazard quotient of ≤ 0.2.

For the chronic assessment, the $PEC_{Adult,\ oral}$ is compared to the chronic adult oral NOAEL (effects to survival or longevity, µg/bee) such that:

$$RQ_{chronic} = PEC_{Adult,oral}/NOAEL_{Adult,oral}$$

The level of concern for the chronic assessment is 1.0.

Where risk quotients exceed the level of concern, further refinement is required – see Section 4.5 below for initial exposure refinement options.

Larvae, oral exposure

Step 1 (Box 3c of the Framework at Appendix A) – Calculate Tier-1 PEC for **Larval oral exposure** *via* brood food.

The exposure estimate for larvae (brood size and success) through diet is provided in the following equation:

$$PEC_{Larvae,oral} = AR\ X\ \left(98\frac{\mu g\ ac}{g}\right) X\ 0.120\frac{g}{day}$$

Where: AR = application rate (kg ac/ha).

The rationale behind the value of 98 µg/g residues is explained in the White Paper (US EPA et al, 2012). Essentially, it equates to the upper-bound (90th percentile) residue for tall grass normalised to an application rate of 1 kg ac/ha and is considered to represent an upper-bound residue value for pollen and nectar. This value is then converted to a dietary dose received by larvae using pollen and nectar consumption rate of 120 mg/day based on 5 day old worker larvae.

Step 2 (Box 4c of Framework) – Calculate Tier-1 Risk Quotients (RQs) for **Larvae oral exposure**.

For an acute assessment the $PEC_{Larvae,\ oral}$ is compared to the larval LD50 (µg/bee; OECD Test Guideline 237) to generate the risk quotient:

$$RQ_{acute} = PEC_{Larvae,oral}/Larval\ LD50$$

The risk quotient is compared to the level of concern for acute exposure of 0.4.

For the chronic assessment, the PECLarvae, oral is compared to the chronic larval oral NOAEL (effects to adult emergence, survival, µg/bee) such that:

$$RQ_{chronic} = PEC_{Larvae,oral}/NOAEL_{Larvae,oral}$$

These toxicity values are obtained from the draft OECD test guideline for repeated exposure, larval toxicity test (see comments under Table 3 above). The level of concern for the chronic assessment is 1.0.

Where risk quotients exceed the level of concern, initial refinement of exposure estimates can be made – see Section 4.5 below for initial exposure refinement options.

4.3 Seed treatment/soil applied

The following methodology needs to be considered in conjunction with the framework shown at Appendix B.

If it is considered at stages 2a and/or 2b of the framework (at Appendix B) that exposure of adult bees and exposure of bee brood is of concern, the following methodology is available for the risk assessment.

In absence of information to indicate otherwise, it is assumed that soil-applied and seed-treated pesticides are systemic and able to be transported to pollen and nectar. Therefore, seed treatments and outdoor application to soils are generally assumed to have a reasonable potential to result in exposure of bees, including both adult and immature stages of bees, to pesticides *via* consumption of contaminated pollen and/or nectar (US EPA et al, 2014).

Seed treatment, Adult oral exposure

Step 1 (Box 3a of Framework at Appendix B) – Calculate Tier-1 PEC for **Adult oral exposure** via pollen and nectar.

In the first instance for seed treatment, pesticide concentrations in pollen and nectar are based on concentrations in leaves and stems of treated plants, taken to be 1 mg/kg (EPPO, 2010). The exposure estimate is calculated as follows:

$$PEC_{Adult,Oral} = 1\frac{\mu g\ ac}{g}\ X\ 0.292\frac{g}{day}$$

Soil applied, Adult oral exposure

Step 1 (Box 3a of Framework at Appendix B) – Calculate Tier-1 PEC for **Adult oral exposure** via pollen and nectar.

In the Tier-1 approach, it is assumed that all chemicals applied as a soil drench, seed treatment, or trunk injection may be systemically transported. This assumption may be refuted using data such as Log Kow and monitoring data. The Brigg's model is currently used to estimate Tier-1 Estimated Exposure Concentrations (EECs) resulting from a soil application. The rationale and formulae for this approach are described in detail in the White Paper (US EPA et al, 2012; Section 3.2.1). Uncertainties surrounding these Tier-1 exposure estimates are explained in Section 4.2.5.1 of US EPA et al (2014).

The exposure estimate from a soil applied substance is calculated as follows:

$$PEC_{Adult,Oral} = Briggs\ EEC\ X\ 0.292\frac{g}{day}$$

Risk characterisation – Adult, oral

Step 2 (Box 4b of Framework) – Calculate Tier-1 Risk Quotients (RQs) for **Adult oral exposure**.

For the acute assessment, the $PEC_{Adult,\ oral}$ is compared to the adult contact LD50 (µg/bee) to generate the risk quotient:

$$RQ_{acute} = PEC_{Adult,oral}/Acute\ LD50_{Adult,oral}$$

The RQ is compared to the level of concern (LOC) of 0.4 for interpretation.

For the chronic assessment, the $PEC_{Adult,\ oral}$ is compared to the chronic adult oral NOAEL (effects to survival or longevity, µg/bee) such that:

$$RQ_{chronic} = PEC_{Adult,oral}/NOAEL_{Adult,oral}$$

The level of concern for the chronic assessment is 1.0.

Where risk quotients exceed the level of concern, further refinement is required—see Section 4.5 below for initial exposure refinement options.

Seed treatment, Larvae oral exposure

Step 1 (Box 3c of the Framework at Appendix B) – Calculate Tier-1 PEC for **Larval oral exposure** *via* brood food.

The exposure estimate for larvae (brood size and success) through diet is provided in the following equation:

$$PEC_{Larvae,Oral} = 1\frac{\mu g\ ac}{g}\ X\ 0.124\frac{g}{day}$$

Where: AR = application rate (kg ac/ha).

Soil applied, Larvae oral exposure

Step 1 (Box 3c of the Framework) – Calculate Tier-1 PEC for **Larval oral exposure** *via* brood food.

In the Tier-1 approach, it is assumed that all chemicals applied as a soil drench, seed treatment, or trunk injection may be systemically transported. This assumption may be refuted using data such as Log Kow and monitoring data. The Brigg's model is currently used to estimate Tier-1 EECs resulting from a soil application. The rationale and formulae for this approach are described in detail in the White Paper (US EPA et al, 2012; Section 3.2.1). Uncertainties surrounding these Tier-1 exposure estimates are explained in Section 4.2.5.1 of US EPA et al (2014). The exposure estimate for larvae (brood size and success) through diet from soil applied substances is provided in the following equation:

$$PEC_{Larvae,Oral} = Briggs\ EEC\ X\ 0.124\frac{g}{day}$$

Where: AR = application rate (kg ac/ha).

Risk characterisation—larvae, oral

Step 2 (Box 4c of Framework of Appendix B) – Calculate Tier-1 Risk Quotients (RQs) for **Larvae oral exposure**.

For the acute assessment where the $PEC_{Larvae,\ oral}$ is compared to the larval LD50 (µg/bee; OECD Test Guideline 237)) to generate the risk quotient:

$$RQ_{acute} = PEC_{Larvae,oral}/Larval\ LD50$$

The LOC for acute exposure is 0.4.

For the chronic assessment, the $PEC_{Larvae,\ oral}$ is compared to the chronic larval oral NOAEL (effects to adult emergence, survival, µg/bee) such that:

$$RQ_{chronic} = PEC_{Larvae,oral}/NOEC_{Larvae,oral}$$

These toxicity values are obtained from the draft OECD test guideline for repeated exposure, larval toxicity test (see comments under Table 3 above). The level of concern for the chronic assessment is 1.0.

Where risk quotients exceed the level of concern, initial refinement of exposure estimates can be made—see Section 4.5 below for initial exposure refinement options.

4.4 Tree trunk injection

US EPA et al (2014) provides Tier-1 methodology for assessing risk from tree trunk injections of pesticides and exposure and risk characterisation formula are described here.

Adult, oral exposure

The exposure estimate for adults (individual survival) through diet is provided in the following equation:

$$PEC_{Adult,Oral} = \left(\frac{\mu g\ ac\ applied\ to\ tree}{g\ of\ foliage}\right) X\ 0.292 \frac{g}{day}$$

For the acute assessment, the $PEC_{Adult,\ oral}$ is compared to the adult contact LD50 (µg/bee) to generate the risk quotient:

$$RQ_{acute} = PEC_{Adult,oral} / Acute\ LD50_{Adult,oral}$$

The RQ is compared to the level of concern (LOC) of 0.4 for interpretation.

For the chronic assessment, the $PEC_{Adult,\ oral}$ is compared to the chronic adult oral NOAEL (effects to survival or longevity, µg/bee) such that:

$$RQ_{chronic} = PEC_{Adult,oral} / NOAEL_{Adult,oral}$$

The level of concern for the chronic assessment is 1.0.

Larvae, oral exposure

The exposure estimates for larvae through diet is provided in the following equation:

$$PEC_{Larvae,Oral} = \left(\frac{\mu g\ ac\ applied\ to\ tree}{g\ of\ foliage}\right) X\ 0.124 \frac{g}{day}$$

For the acute assessment where the $PEC_{Larvae,\ oral}$ is compared to the larval LD50 (µg/bee; OECD Test Guideline 237)) to generate the risk quotient:

$$RQ_{acute} = PEC_{Larvae,oral} / Larval\ LD50$$

The LOC for acute exposure is 0.4.

For the chronic assessment, the $PEC_{Larvae, oral}$ is compared to the chronic larval oral NOAEL (effects to adult emergence, survival, µg/bee) such that:

$$RQ_{chronic} = PEC_{Larvae,oral}/NOEC_{Larvae,oral}$$

These toxicity values are obtained from the draft OECD test guideline for repeated exposure, larval toxicity test (see comments under Table 3 above). The level of concern for the chronic assessment is 1.0.

4.5 Refine Tier-1 exposure estimates

Box 6 of the Framework—Refine Tier-1 Exposure assessment, for example, using available crop residues data; Recalculate Risk Quotients.

Contact exposure

Box 6 of Framework: Refine Tier-1 Exposure assessment; recalculate RQs.

> EFSA (2013) considers whether it is possible to refine the contact exposure estimate; for example, it may be relevant to consider the spray drift on to the field margin or adjacent crop. If this is considered appropriate, the RQ should be recalculated, based on the fraction of the dose deposited on foragers visiting plants in the field margin or an adjacent crop.
>
> For Australian assessments, the fraction of drift should be calculated from the current spray drift risk assessment tools, AgDRIFT for ground boom/orchard sprays and AgDISP for aerial application. These assessments should be conducted in accordance with the APVMA's current Spray Drift Risk Operating Principles.[10]
>
> Appendix N, Section 2 of EFSA (2013) provides a considered approach to refining the exposure assessment for spray applications.

If the risk quotients (Box 7 a, b, c of the Framework) using the refined Tier-1 exposure calculations still exceed the level of concern, see Section 5 below.

Downwind buffer zones

While Australia does not calculate terrestrial downwind buffer zones for the protection of insect populations, buffer zones can be calculated with respect to determining a downwind distance between the treated field and location of bee hives.

The allowable downwind deposition (referred to here as the 'Regulatory Acceptable Level', or RAL) should be calculated from the current spray drift risk assessment tools, AgDRIFT for ground boom/orchard sprays and

[10] archive.apvma.gov.au/archive/spray_drift/op_principles.php

AgDISP for aerial application. These assessments should be conducted in accordance with the APVMA's current Spray Drift Risk Operating Principles.

The exposure should be based on a downwind width of area average of 3 m with the RAL on the adult contact LD50 and applying a level of concern of 0.4 in accordance with the US EPA et al (2014) level of concern for an acute assessment.

The RAL, which will determine the downwind buffer zone, needs to be calculated through re-arranging the exposure and risk quotient formulae described for acute contact risk in Section 4.2 above as follows, given the acceptable RQ = 0.4:

1) $0.4 = PEC_{Contact}/Acute\ LD50_{Contact}$

2) $PEC_{Contact} = 0.4\ x\ Acute\ LD50_{Contact}$

3) $AR\ X\ 2.4\ \mu g\ ac/bee = 0.4\ x\ Acute\ LD50_{Contact}$

4) $AR = (0.4\ x\ Acute\ LD50_{Contact})/(2.4\ \mu g\ ac/bee)$

In these calculations, the application rate (AR) becomes the RAL. For example, an LD50 = 50 µg/bee results in a RAL of 8.3 g ac/ha while a much more toxic substance, for example an LD50 = 0.5 µg/bee results in a RAL of 0.083 g ac/ha.

Oral exposure

Initial refinements are made for the exposure calculations with respect to consumption of food and pollen. The initial refinement provided in US EPA et al (2014) uses measured pesticide concentrations in pollen and nectar of treated crops. This document provides guidance relating to quantifying residues of pesticides in pollen and nectar using pesticide specific studies, but does not provide available data on measured levels. However, Appendices 3 and 4 from the White Paper (US EPA et al, 2012) provide details from a range of experiments with different crops and active substances, reporting residue levels in different matrices which may be used to inform pollinator exposure at this level of assessment.

Appendix F in EFSA (2013) provides a table of the compiled residue unit dose (RUD)[11] values from foliar spray applications, normalised to an application rate of 1 kg/ha. The information and methods used to derive these values are described in that document.

Such measured data may then be used to further calculate exposure levels for other castes of bees using their food consumption rates. Suggested food consumption rates are summarised in the following table; further data supporting these values is described in US EPA et al (2012; 2014).

[11] Residue Unit Dose is defined as the mass of substance per mass of insect after spraying a dose of 1 kg/ha.

Table 10: Estimated food consumption rates of bees (US EPA et al, 2014)

LIFE STAGE	CASTE (TASK IN HIVE)[1]	AVERAGE AGE (D)[1]	DAILY CONSUMPTION RATE (mg/d)			
			JELLY	NECTAR[2]	POLLEN	TOTAL
Larval	Worker	1	1.9	0	0	1.9
		2	9.4	0	0	9.4
		6	19	0	0	19
		4	0	60[3]	1.8[4]	62
		5	0	120[3]	3.6[4]	124
	Drone	6+	0	130	3.6[5]	134
	Queen	1	1.9	0	0	1.9
		2	9.4	0	0	9.4
		3	23	0	0	23
		4+	141	0	0	141
Adult	Worker (cell cleaning and capping)	0–10	0	60[6]	1.3–12[7, 8]	61.72
	Worker (brood and queen tending, nurse bees)	6–17	0	113–167[6]	1.3–12[7, 8]	114–179
	Worker (comb building, cleaning and food handling)	11–18	0	60[6]	1.7[7]	62
	Worker (foraging for pollen)	>18	0	35–52[6]	0.041[7]	35–52
	Worker (foraging for nectar)	>18	0	292[3]	0.041[7]	292
	Worker (maintenance of hive in winter)	0–90	0	29[6]	2[7]	31
	Drone	>10	0	133–337[3]	0.002[3]	133–337
	Queen (laying 1500 eggs/day)	Full lifestage	525	0	0	525

(1) Winston (1987). (2) Consumption of honey is converted to nectar-equivalents using sugar contents of honey and nectar. (3) Calculated as described in US EPA et al *(2014; 2012). (4) Simpson (1955) and Babendreier* et al *(2004). (5) Pollen consumption rates for drone larvae are unknown. Pollen consumption rates for worker larvae are used as a surrogate. (6) Based on sugar consumption rates of Rortais* et al *(2005). Assumes that average sugar content of nectar is 30 per cent. (7) Crailsheim* et al *(1992; 1993). (8) Pain & Maugenet (1966).*

The US EPA applies the 'Bee-REX' tool to calculate dietary exposure values and associated risk quotients for larvae of different ages, adult workers with different tasks (and associated energetic requirements) and the queen. This is accomplished using the above food consumption rates. This Bee-REX tool is not available in Australia,

however, Tier-2 exposure values for residues entering the hive can be calculated through a residue intake rate (RI) following the EFSA (2013) methodology (Appendix N of that document). Briefly, the RI is expressed in µg/day and calculated as:

$$RI = \frac{PEC_{Pollen} C_{pollen} + PEC_{nectar} C_{nectar}}{1000}$$

Where PEC_{Pollen} and PEC_{Nectar} are the predicted environmental concentrations (mg/kg) in pollen and nectar, respectively, and C_{pollen} and C_{nectar} are the consumption rates of pollen and nectar of a bee species (mg/day). Division by 1000 is needed because the numerator of the above equation generates RI in 10^{-6} mg/day, which is divided by 1000 to convert to µg/day.

The EFSA methodology (EFSA, 2013) described in Appendix 3 is considered a screening level assessment by EFSA. Their first-tier risk assessment for refining exposure from the consumption of pollen and nectar is undertaken through consideration of all appropriate routes of exposure, including:

- risk from foraging on the treated crop
- risk from foraging on weeds in the treated field
- risk from foraging in the field margin
- risk from foraging on an adjacent crop
- risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops.

Revised exposure calculations for all the relevant scenarios above are still calculated using shortcut values (as described for EFSA methodology in Appendix 3) for acute adult, chronic adult and larvae—refer to Appendix J of EFSA (2013) for the refined shortcut values and rationale supporting them.

If the risk quotients (Box 7 a, b, c of the Framework) using the refined Tier-1 exposure calculations still exceed the level of concern, see Section 5 below.

4.6 Consider risk mitigation options, uncertainties and other lines of evidence

Box 8 of the Framework – Consider risk mitigation options, uncertainties and other lines of evidence.

If risks are identified from Tier-1, the risk assessor should consider the uncertainties associated with risk estimation, information from other lines of evidence, and the impact of any risk mitigation options identified for the pesticide of concern. These risk mitigation options may include reductions in application rates and restriction of application methods, and recalculation of Tier-1 risk estimates as a result of reduced environmental loading. Restrictions on the timing of pesticide applications and crop species may also be considered to minimise exposure to bees. The risk assessor should also consider whether information on pesticide exposure and effects collected using Tier-2 studies are needed (US EPA et al, 2014).

5 RISK ASSESSMENT—HONEY BEES, TIER-2

This second tier of assessment can be applied in risk quotients in Tier-1 exceed levels of concern and additional refinement is required.

5.1 Effects, Tier-2

Box 10 of the Framework (Appendix A and B) – Evaluate Tier-2 Exposure and colony level effect results. Consider uncertainties and other lines of evidence. Do results indicate a risk?

Tier-2 studies may be used to identify more targeted risk mitigation options than those that could be identified based on Tier-1 data. Measured residues in pollen and nectar from these studies may also be used to refine risk estimates in Tier-1. Tier-2 effect studies characterise pesticide effects at the whole-colony level and therefore, reduce uncertainty associated with extrapolating effects on individual bees under laboratory conditions (Tier-1 toxicity studies) to effects on the colony. It is important to recognise that Tier-2 effect studies are conducted under semi-field conditions in which the high-end exposure at the colony level is expected. In Tier-2 studies other stressors may be present and potential compensatory mechanisms colony may occur within the colony. Tier-2 studies should be designed to address potential uncertainties identified in the Tier-1 assessment or elsewhere (e incident reports). Unlike Tier-1, characterisation of risk in Tier-2 does not involve the calculation of RQ values per se. Rather, risks at the colony level are usually characterised in relation to pesticide application rate and/or measured residue levels. Interpretation of such studies investigating whole-colony effects is often much more complex than interpreting Tier-I studies, and relies on comprehensive considerations of whether adverse effects are likely to occur at the colony level (US EPA et al, 2014).

A summary of Tier-2 studies and their strengths and limitations is described in Table 5 (above).

The decision to move to more refined effects testing and to transition from laboratory-based studies with individual bees (Tier-1) to colony-based studies (Tier-2 or Tier-3) depends on whether Tier-1 LOCs are exceeded, the availability of data, and the nature of uncertainties that warrant further testing. The Tier-2 studies are typically considered ‘semi-field’ studies where small colonies (referred to as nuclei colonies or ‘nucs’)[12] are enclosed in tunnels, along with pesticide-treated crops. Tier-2 studies may also include feeding studies in which whole colonies are tested; however, the colonies are not confined to enclosures. Typical semi-field studies are usually conducted under conditions that represent the worst-case exposure scenario of proposed uses to the entire colony (over the duration of the study) or designed to address specific uncertainties with respect to effects on the colony. Feeding studies, on the other hand, are usually conducted with diets spiked with known concentrations of test chemical, using colonies that are not confined to enclosures (free-foraging bees) (US EPA et al, 2014).

The Tier-2 study designs may be amenable to additional treatment levels and replication, thus facilitating the quantification of an application rate-response (tunnel study) or dose-response (feeding study) relationship at the colony level and subsequent determination of a NOAEC. This information may be particularly useful and

[12] Nuclei colonies have been characterised as consisting of approximately 3000 brood cells with brood in all stages, one good comb with honey and pollen and approximately 6000 worker bees; the ratio of brood to food (pollen/nectar) should not exceed 4:1 (OECD, 2007).

transferable from the test crop/concentration to other crops where residue concentrations in pollen and nectar are available in conjunction with associated application rates (US EPA et al, 2014).

Guidance regarding Tier-2 study designs and interpretation are found in the various source documents in the following sections:

- US EPA et al, 2014: Section 3.2.5. p 24; Tier-2 Effects Characterisation
- White paper (US EPA et al, 2012): Section 4.2, p 116; Tier-2 Effects Assessment
- EFSA: Appendix O – Effect Studies: Protocols, guidance and guidelines for honey bee, bumble bee and solitary bee.

6 RISK ASSESSMENT—HONEY BEES, TIER-3

A summary of Tier-3 studies and their strengths and limitations are described in Table 6 above. The following discussion on Tier-3 effects characterisation is from US EPA et al, 2014:

> Tier-3 field studies are usually highly complex and require a high level of effort to design and conduct so as to address specific sources of uncertainties and potential risks identified in lower tiers. Because of the length and complexity of these studies, other factors affecting colony survival (eg, disease, pests, nutrition) may impact the successful completion and interpretation of these studies. As with any field study, the design and conduct of such studies is crucial to their interpretation and utility in risk assessment. Similar to risk characterization at Tier-2, risk characterization at Tier-3 considers multiple lines of evidence available from lower Tiers and other information sources (eg, open literature) that meet the respective Agency's standard for inclusion in risk. Risk assessment conclusions are made based on the weight of evidence, available risk mitigation options, and uncertainties in the available data and methods.
>
> While guidance is available for field pollinator testing (eg, OCSPP Guideline 850.304021, EPPO 170), this information is relatively generic and is intended to provide information on study design elements that should be considered in conducting field pollinator studies. Additional general guidance is provided in the Field testing for Terrestrial Wildlife guidance document (OCSPP Guideline 850.2500).

More detailed guidance on Tier-3 studies is available in the source documents as follows:

- US EPA et al, 2014: Section 3.2.6. p 27; Tier-3 Effects Characterisation
- US EPA et al, 2014: Appendix 4. Tier-3 Field Study Design Considerations, p 57
- White paper (US EPA et al, 2012): Section 4.3, p 127; Tier-3 Effects Assessment
- EFSA – Appendix O – Effect Studies: Protocols, guidance and guidelines for honey bee, bumble bee and solitary bee.

7 RISK ASSESSMENT—NATIVE BEES AND OTHER INSECT POLLINATORS

The goal of protecting pollinator biodiversity is an ecological protection goal in that it aims to protect populations of pollinator species that contribute to the health of the wider environment. While the APVMA's 2013 regulatory workshop recognised that such species may be exposed in agricultural and horticultural crops, the primary issue for native bees and other pollinators relates to biodiversity and their general ecological significance. Therefore, the assessment for these species should focus on the off-crop environment.

The workshop discussed assessment methodology with respect to native bees and other pollinators in the context of the non-target arthropod assessment, and the latest guidance available from ESCORT 3 (Alix et al, 2012).

ESCORT 3 describes the outcomes of the third 'European Standard Characteristics of Beneficials Regulatory Testing' workshop held in March 2010. While that group is based in Europe, they note that the developments and recommendations they proposed also addressed questions and concerns raised in countries beyond Europe, and that the guidance generated in ESCORT 3 would be readily transferrable into non-European areas.

In terms of the off-crop environment, ESCORT 3 gave some specific consideration to pollinators other than honey bees. The wording from this document (Alix et al, 2012) with respect to pollinators other than honey bees is paraphrased as follows:

> If risk assessment for NTAs (non-target arthropods) also needs to consider "pollinators other than honey bees," then oral routes of uptake or exposure and modes-of-action (MoAs) may also need to be better reflected in the standard suite of NTA tests. However, we recognised that the honey bee acute oral test does provide information on oral toxicity .When the results of this oral honey bee toxicity data are considered alongside the lower-tier NTA data, this should provide an initial understanding of the potential risk to other pollinators.
>
> However, where oral routes of uptake or exposure and MoAs including systemically active products need to be considered further, then higher tier assessment for "pollinators other than honey bees" should be included. Where information suggests the existing tests on honey bees or NTAs do not reflect appropriate routes of uptake, then additional testing may be necessary. Because pollinators often move freely between in- and off-crop habitats, systemic seed treatments may still need to be assessed for off-crop risk. Also, such pollinators may be exposed to dust from the drilling of treated seed.

With regard to assessment for pollinators other than honey bees, ESCORT 3 went on to recommend:

> The current lower-tier NTA testing scheme, along with the standard honey bee scheme, should provide an adequate screening tool for other pollinators. Where oral routes of uptake or exposure (including systemic) and MoAs need to be considered further, then higher-tier assessment for "other pollinators" should be included.

The use of NTA data as surrogate toxicity data for non-*Apis* pollinators was also considered within the overall risk assessment framework outlined at the SETAC Pellston Workshop and described in Fischer and Moriarty (2011).

The APVMA's 2013 regulatory workshop discussed in some detail the applicability of using NTA toxicity data as a surrogate for native bees and other insect pollinators, including consideration of how relevant the wide range of tests for non-target arthropods (for example, parasitic wasps, predatory mites, mayflies, ladybird beetles, predatory bugs) may be to these organisms.

Non-*Apis* bees (including native bees) are not used for commercial pollination activities in Australia, so the non-*Apis* assessment in Australia is directed primarily as an off-field assessment; consequently, it was agreed that the use of NTA data (mainly relating to mortality and reproduction), along with inclusion of *Apis* toxicity test data would provide a suitable basis for assessing risk to native bees and other insect pollinators. This fits in with the approach described in ESCORT 3 and should be adopted in Australian assessments at least as a first-tier assessment. However, both in-field and off-field exposure estimates should still be used in the assessment for native bees and other insect pollinators, since they may forage in treated crops.

If *Apis* toxicity data are applied as a surrogate for solitary bees (as is expected in the case on Australian native bees), an assessment factor of 10 is applied in the EFSA (2013) guidance; this assessment factor should be adopted in Australian assessments.

8 WEIGHT-OF-EVIDENCE AND UNCERTAINTY

Both the North American and European guidance documents recognise the importance of addressing uncertainties in the risk assessment. Assessors should consider the guidance provided in both these source documents when considering the outcomes of the risk assessment.

8.1 European guidance

Risk characterisation and weight-of-evidence assessment

EFSA (2013) recommends the following approach (Section 6.2 of that document) is taken regarding a weight-of-evidence assessment:

- Consider all relevant lines of evidence, including the first tier assessment. Retention of the first tier assessment is appropriate in all cases, as it is relevant to consider whether it was borderline or failed by a large margin.
- Evaluate the uncertainties associated with each line of evidence. This should be done by applying the approaches described in the preceding section (that is, Section 6.1 in the EFSA 2013 guidance document) to each line of evidence separately. The characterisation of overall uncertainty for each line of evidence is then used in the weight-of-evidence assessment, as in principle the weight given to each line of evidence should be proportional to its certainty.
- Form overall conclusions by using expert judgement to combine all lines of evidence, weighted according to their certainty, and give more weight to the most certain, but also take due account of the less certain. High certainty implies high weight. If one line of evidence implies a much narrower range for the risk than another line of evidence (i.e. higher certainty), then the true risk is most likely to fall inside the range of the former.
- Be sure to take full account of the uncertainties and to include a fair description of the range of possible outcomes in the final risk characterisation. Identify the outcome that is considered most likely, but do not give it more emphasis than is justified by the evidence.
- If different lines of evidence conflict (eg, a high ETR but no effects in a field study), this should be considered a form of uncertainty. No line of evidence should be completely discounted unless it is wholly invalid or irrelevant. Instead, as stated above, each line of evidence should contribute to the overall conclusion in proportion to its certainty.
- If the overall characterisation of risk is expressed qualitatively, choose words very carefully to describe the outcome and its uncertainty as clearly as possible. For example the phrase ‘on balance’ is often used to focus on one of several possible outcomes. An example is: ‘On balance, it is concluded there will be no mortality’. This type of statement is not appropriate, because it fails to communicate the degree of certainty. ‘On balance’ could mean 51 per cent certainty, or 99 per cent.
- A weight-of-evidence assessment is inevitably subjective. Different assessors may vary in their weighing of the evidence, especially when uncertainty is high. Therefore, it is essential to document the assessment in detail, including the outcome and uncertainty for each lines of evidence considered, and to explain how they were combined to reach conclusions about the overall outcome and its uncertainty.

EFSA (2013) recommends a systematic tabular approach to documenting the weight-of-evidence and provides examples in their guidance document.

Uncertainty

A full discussion on assessing uncertainty in EFSA (2013) is found in Chapter 6 (p 59 of that document) and Appendix V (p 242 of that document). The following summarises the uncertainty analysis described in EFSA (2013).

All uncertainties affecting an assessment should be considered at least qualitatively. To reduce the risk of overlooking important uncertainties, it is recommended that each part of the assessment (eg, different lines of evidence, different inputs to calculations, etc.) be systematically considered and all of the sources of uncertainty listed, together with a description of the magnitude and direction of their potential influence on the expected level of impact. As well as evaluating each individual source of uncertainty, it is also essential to give an indication of their combined effect. It is recommended that a tabular approach be used to facilitate and document this process. To assist in adopting a tabular approach, EFSA (2013) provides example tables in Chapter 6, and worked examples in Appendix V.

To help characterise uncertainty, EFSA (2013) groups the three main types as follows:

- Qualitative methods: using words to describe the certainty of an outcome, or to describe how different the true outcome might be compared with an estimate.
- Deterministic methods: generating deterministic quantitative estimates of impact for a range of possible scenarios. This shows the range of possible outcomes (eg, a range of ETRs) and can be accompanied by qualitative descriptions of their relative probabilities (traditional ‘worst-case’ assessments are an example of this).
- Probabilistic methods: these give numeric estimates of the probabilities of different outcomes (Luttik et al, 2011). These probabilities may be estimated statistically (eg, when quantifying measurement or sampling uncertainty, or as outputs from probabilistic modelling). However, they may also be estimated subjectively, by expert judgement.

It is then recommended in summary that:

- Every refined risk assessment should be accompanied by at least a qualitative evaluation of the uncertainties affecting it, using a systematic tabular approach. In assessments with multiple lines of evidence, the uncertainties affecting each line of evidence should be evaluated separately.
- In cases in which qualitative evaluation of uncertainty is not sufficient to determine whether it is clearly established that no unacceptable impact will occur, the assessor may either (a) seek further data to reduce the uncertainty or (b) refine the evaluation of the existing uncertainties using quantitative methods (which can be either deterministic or probabilistic).
- Every refined risk assessment should conclude with an overall characterisation of risk, in terms relevant for decision-making. It is recommended to begin with the consideration of whether the evidence makes any mortality or reproductive effects unlikely. Where this is not satisfied, attention should turn to characterising the levels of mortality and reproductive effects that may occur, and using this to evaluate whether there is a high

certainty that the magnitude of effects on colonies should not exceed 7 per cent reduction in colony size and that forager mortality should not be increased compared with controls by a factor of 1.5 for six days or a factor of 2 for three days or a factor of 3 for two days.

- The overall characterisation of risk should be derived by a qualitative weight-of-evidence assessment considering all relevant lines of evidence (see above) and their uncertainties using a systematic tabular approach. If the overall characterisation is expressed qualitatively (in words) rather than quantitatively, great care should be taken to describe the outcome and its uncertainty as clearly as possible.

The first tier assessment should always be included as one of the lines of evidence and given appropriate weight (this will be higher for acute risks of sprayed pesticides than for other types of assessment).

8.2 US EPA et al (2014)

The North American guidance document deals with uncertainties in the risk assessment in Section 4.2.5 (p 36) as part of a wider chapter dedicated to risk description. The risk description phase is stated to provide an opportunity to discuss additional lines of evidence and uncertainties regarding the potential risks to bees beyond the risk quotients calculated in the risk estimation.

Weight-of-evidence

This is generally considered in the North American guidance as ‘other lines of evidence’.

In agreement with the European guidance, it noted that in characterising uncertainty, the risk assessor should consider the full weight-of-evidence, and application of a weight-of-evidence analysis is an integrative and interpretive process taking into account all relevant scientific information. The analysis should consider whether the existing data provide relevant, robust and consistent evidence (for example, within and among the outcomes of laboratory and semi-/full-field studies) that a chemical has the potential to adversely affect bees and at what level of biological organisation (individual bees or at the colony level) and under what conditions (what application rate, exposure duration).

As described in the White Paper (US EPA et al 2012), the risk assessment process is intended to integrate multiple lines of evidence. The process used for evaluating these multiple lines of evidence has not been specifically articulated for honey bees; however, it has been described in a draft guidance developed for evaluating the weight of evidence for the Endocrine Disruptor Screening Program (EDSP) Tier-1 screening to identify candidate chemicals for Tier-2 testing (USEPA 2010). In the draft guidance, it indicates that the body of available data is taken into account for consistency, coherence, and biological plausibility. This analysis not only applies to the outcome of guideline studies but also other scientifically relevant information, which in the case of risk assessments for bees would include targeted residue monitoring studies, open literature studies, and incident reports. More specifically, the evaluation of individual studies includes the characterisation of:

- quality of data and the extent to which effects can be replicated within a laboratory and across different laboratories
- nature of the effect(s) seen in the study(ies) (for example, were the effects seen in studies persistent or transient changes?; were they sub-lethal changes or adverse outcomes?)
- dose and time dependent changes, if available

- strengths and limitations of results
- number and type of effects induced and magnitude, and severity of effects
- consistency, pattern, range, and interrelationships of effects observed across studies, species, strains, and castes/sexes
- conditions under which effects occur (for example dose, route, duration)
- understanding of MOA and biological plausibility of responses, and
- specificity and sensitivity of the effect(s).

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

Uncertainties

US EPA et al (2014) observes that the primary sources of uncertainties for the risk assessment are related to estimating pesticide exposures to bees and effect of those exposures to bees. With respect to dietary exposure, the first source of uncertainty may be related to the extent to which the amount of food consumed by bees for the Tier-1 exposure estimate represents pesticide concentration in bee food sources. With respect to contact exposure, there is uncertainty as to the extent that residues on leaves and even soil may be available to bees for uptake. There is also uncertainty as to the extent to which bees may be exposed to pesticide residues through various sources of water, including puddle and plant exudates, and whether that water is ingested, used to dilute honey, or used to cool the colony. The second source of uncertainty is related to differences in bee biology and their foraging behaviours that may directly impact exposure routes and the extent to which bees forage on the treated crop. All these sources of uncertainty may be reflected in the study designs at different tiers.

This North American guidance provides discussion on uncertainties in the following areas [both the overall guidance document (US EPA et al, 2014) and the White Paper (US EPA et al, 2012)], which assessors should be aware of:

- Tier-1 exposure estimates
- use of residue data
- agronomic practices
- pollination biology
    - bee visitation to the flowers of the ornamental, forestry tree or crop
    - harvest period of the crop
    - bloom period of the crop
    - pollen *versus* nectar as the food source of the bees
    - bee diversity.
- differences in bee life history
- differences in pests/pathogens/nutrition/management
- uncertainty in study designs.

9 SUB-LETHAL EFFECTS

European guidance

An illustrative scheme for considering sub-lethal effects is provided in EFSA (2013), Appendix W, p 247. Sub-lethal doses can be defined as a fraction of the LD50. Sub-lethal doses are often an order of magnitude below lethal doses (below LD50/10). Such sub-lethal effects have been reported for a variety of endpoints including biochemical, physiological and behavioural endpoints (cholinesterase activity, survival, development, longevity, locomotion or mobility, navigation or orientation, feeding behaviour and learning performance). The integration of sub-lethal dose effects can provide a better understanding of short-term and long-term effects on honey bees, bumble bees and solitary bees.

The following issues were identified by the experts developing the European Guidance Document, which need to be understood before sub-lethal effects can be fully integrated in a risk assessment scheme:

1. Protection goals and trigger values in the first tier.
2. Interpretation of sub-lethal effects in terms of effects on the colony.
3. Exposure assessment goals for the homing flight study.
4. Interpretation of effects observed in the homing flight study.

Further context for these different issues is provided in EFSA (2013).

North American guidance

The risk description on sub-lethal effects is described in US EPA et al (2014) (p 35) within the Risk Description chapter. That guidance states that, in addition to the measurement endpoints that have direct linkages to assessment endpoints and that were used quantitatively in the risk estimation section of the risk characterization, the risk description should also include, if available, sub-lethal endpoints such as behavioural effects and proboscis extension reflex that are associated with the chemical under evaluation.

However, it is further acknowledged that these sub-lethal endpoints often lack information on subsequent effects on survival, growth and reproduction. Further, although the US Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) recommended an increased use of sub-lethal endpoints, until suitable linkages have been developed between sub-lethal measurement endpoints to assessment endpoints, their use in pollinator risk assessment should remain qualitative.

10 RISK MITIGATION—POLLINATOR PROTECTION STATEMENTS

Currently in Australia, there is a wide variety of bee protection statements, with differences between products of different active constituents and even between products containing the same active constituent. An APVMA label review in 2013 identified forty seven different bee protection statements. Products could contain between one and five of these statements, in some cases, under more than one protection statement heading.

The issue of label statements including the current wide variety and lack of consistency was considered at the 2013 APVMA workshop, on 'Pesticides and the Health of Insect Pollinators', together with issues such as use patterns for which statements would be required. It was generally agreed that protection statements are required to address both hazard and risk pertaining to an active constituent and its use pattern, however, there was insufficient time during the workshop to consider a potential suite of statements or define the list of use situations where statements were required.

One important outcome from the workshop was the need to build label statements into the risk assessment framework. The following section addresses this issue.

Following the workshop, and in consultation with staff in the Department of the Environment, the following approach with respect to pollinator protection statements was agreed.

10.1 Protection statement headings

The APVMA's 2013 label review identified ten different label signal headings under which bee protection statements may be found. Given the need to undertake more comprehensive risk assessments on bees and other insect pollinators, it is recommended that protection statements are placed on product labels under an individual protection statement heading as follows:

PROTECTION OF HONEY BEES AND OTHER INSECT POLLINATORS

Currently, bee protection statements are found under headings related to livestock protection or environmental protection. This makes it difficult for users to quickly find instructions relating to protection of bees and pollinators. The use of a separate unique heading will negate this difficulty. This is consistent with recent international approaches. For example, the US EPA has a new advisory box on their pesticide labels designed to protect pollinators with a 'Protection of Pollinators' section.[13]

10.2 Protection statements

The suggested protection statements have been considered in the context of the Tier-I risk assessment framework for honey bees described in Section 3.2 above. Where data have been provided and allow for a higher-tier assessment, there may be the need to refine label statements. These should be done on a case-by-case basis, and in consultation with applicants.

[13] The US EPA bee advisory box designed as part of their strengthened label requirements for pollinator protection is available at: www.epa.gov/opp00001/ecosystem/pollinator/bee-label-info-graphic.pdf

Inclusion of a label statement relating to the hazard of the active constituent or product to bees is appropriate. It was noted that the US EPA applies two ratings, 'Highly toxic to bees' and 'Toxic to bees' where the acute LD50 is ≤ 2 µg/bee and > 2 and ≤ 11 µg/bee respectively.

For some years, the Australian Department of the Environment has been applying a hazard classification system for bee toxicity based on that described in Mensink et al (1995). The three highest hazard classifications in this scheme are:

LD50 <0.1 µg/bee	Highly toxic
LD50 0.1–1.0 µg/bee	Toxic
LD50 1.0–10 µg/bee	Moderately toxic

It is appropriate to maintain the rating system that has been used historically in Australia; these classifications are applied to invoke the following hazard statements on the label:

Hazard-based label statements

Table 11: Hazard statements based on adult acute oral and contact toxicity results

ADULT ORAL AND/OR CONTACT LD50	REQIRED HAZARD STATEMENT
>10 µg/bee	No statement required
1.0–10 µg/bee	*Moderately toxic to bees*
0.1–1.0 µg/bee	*Toxic to bees*
<0.1 µg/bee	*Highly toxic to bees*

Systemic compounds: Apiarists requested the APVMA to include information on product labels if the active constituent has a systemic mode of action. The following statement is suggested.

Table 12: Statement for active constituents which have a systemic action

CONSIDERATION	SUGGESTED STATEMENT
Systemic compounds	*<ACTIVE> has a systemic action.*

Risk-based label statements

Even though a substance may be hazardous its use may be acceptable if exposure is unlikely to result in levels which are toxic to the organism. The following statements are designed to communicate the potential for risk to bees. Where a hazard statement is required, these risk-based statements should follow the hazard statement:

Table 13: Risk statements based on risk quotients (RQs)

RISK ASSESSMENT OUTCOME	SUGGESTED STATEMENT (TO BE ADDED AFTER HAZARD STATEMENT)
Hazardous, but RQ <LOC	*The use pattern as per the Directions for Use is not expected to result in exposure to bees.*
Adult RQ ≥ LOC	*A risk is identified for bees foraging in the crop to be treated or in hives and non-target areas which are over-sprayed or reached by spray drift.*
Immature bees (bee brood) RQ ≥ LOC	*Bee brood development may be harmed by exposure to residues transported into the hive by foraging bees, overspray or spray drift.*

These risk-based statements correspond to Stage 5 or Stage 7 (a, b and c) of the Risk Assessment Framework described in Appendices A and B.

In cases in which higher-tier studies are available, risk based statements may be able to be further refined. Australia has not routinely addressed chronic toxicity of pesticides to bees, either individually or at colony level, through the use of label statements. The need for such statements should be considered on a case-by-case basis and in consultation with the applicant. The following risk statements can be applied based on the outcomes of Tier-2 tests (exposure and colony level results) or Tier-3 field study results:

Table 14: Risk statements based on higher-tier studies

RISK ASSESSMENT OUTCOME	SUGGESTED STATEMENT (TO BE ADDED AFTER HAZARD STATEMENT)
No chronic/colony effects observed	No further statement required
Chronic/colony effects observed—example statements:	**Foliar applied**: *The persistence of residues and available long-term toxicity data suggests the potential for long-term effects on honey bee larvae and colonies* **Soil applied/seed treatment**: *The persistence of residues and expression of [*substance*] in nectar and pollen [add 'extra-floral nectaries', if relevant] suggests the potential for long-term effects on honey bee larvae and colonies*

These risk-based statements correspond to Stage 5 or Stage 7 (a, b and c) of the Risk Assessment Framework described in Appendices 1 and 2.

Risk management statements

Subject to the outcome of the risk assessment, if both a hazard and a potential risk to bees (adults and/or colony) have been identified, risk management statements will also be required. These risk management statements are linked to the residual toxicity characteristics of the compound.

In this regard, it is useful to consider a definition of a 'pollinator area' and the relationship with managed bee hives. The APVMA has defined a 'pollinator area' as an area which includes managed bee hives. For regulatory purposes, this definition only applies when the manager of those bee hives has provided notification regarding their location to the chemical user, or the person the chemical user is applying agricultural chemical product(s) on

behalf of, at least 48 hours prior to application of the agricultural chemical product/s. Whilst notification can be made directly (in writing or verbally), the use of the BeeConnected website (http://beeconnected.org.au/) or smartphone app is acceptable and recommended.

The following table (**Error! Reference source not found.**) provide suggested statements. Such statements need o reflect, as best as possible, what is known about the toxicity of pesticide residue in/on the treated crop, the persistence of these residues, and the mobility of these residues ie. will residues of concern be found in nectar and pollen after foliar application or after soil or seed treatment applications? In relation to these statements, Table 15 provides a further advice statement for pesticides which have a known systemic action.

Table 15: Risk management statements where a potential risk is identified

CONSIDERATION	SUGGESTED STATEMENTS
1) No concerns about persistence of residues have been identified. Statements relating to direct pollinator contact with spray or spray drift may be chosen from the following:	*DO NOT spray while bees are actively foraging on and around the treatment area.* *If there is potential for managed hives to be affected by the spray or spray drift, notify beekeepers 48 hours before spraying to move hives to a safe location.* *The risk to bees may be reduced by spraying in the early morning or late evening while bees are not foraging, provided that surface temperature inversion conditions are not present.*
2) In addition to the above, if residues of the active constituent are known to, or are likely to persist in the environment, include one of the following statements:	*Residues may remain at levels toxic to bees for (X)* days following application. DO NOT apply where bees from managed hives are known to be foraging, and crops, weeds or cover crops are in flower at the time of spraying, or are expected to flower within (X) days,*

*X is calculated using the foliage half-life (in the absence of relevant data, the default half-life is 10 days) and taking into account the acute adult contact LD_{50}. $X = LN(LD50\, x\frac{0.17}{AR})/(-\mathrm{k})$

NOTE: It may be desirable to have crop-specific instructions within the 'Directions for Use' table. If this is the case, the following additional statement should be added to the 'Protection of Honey Bees and Other Insect Pollinators' section of the label:

> **Statement**: *In order to protect insect pollinators, refer to the 'Directions for Use' for crop-specific application restrictions.*

10.3 International approaches to risk mitigation statements

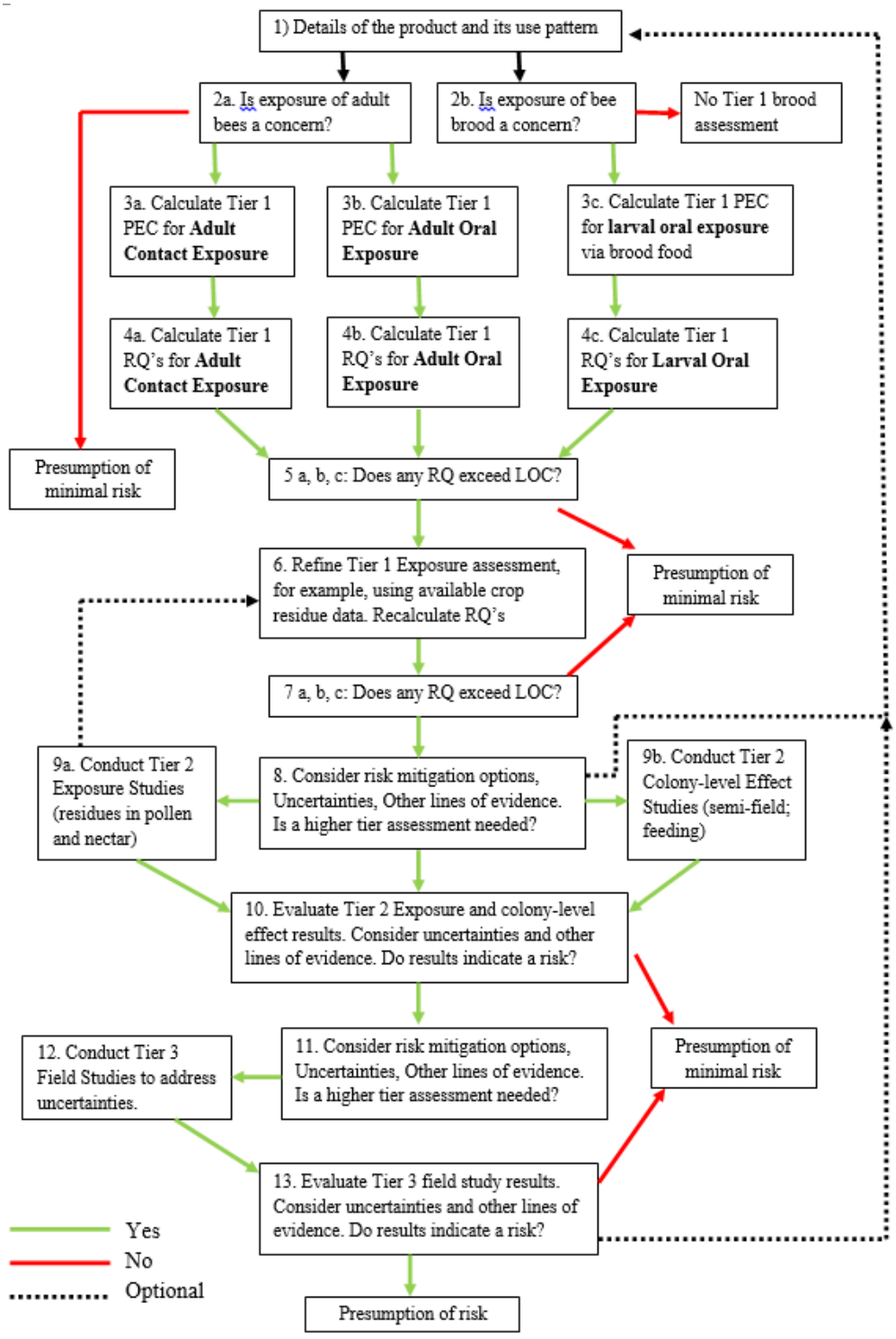
The US EPA provides precautionary label statements for protection of pollinating insects in their Label Review Manual[14]. Specifically, pollinating Insect hazard statements are found in Section B, Table 2 of Chapter 8 (Environmental Hazards) of this document.

EFSA (2013) devotes Chapter 11 (Risk mitigation options) to potential label statements designed to mitigate risk. The chapter includes useful definitions and considers a wide range of situations where mitigation statements may be required such as within the treated crop, within treated weeds in the field, field margin, adjacent crops, succeeding crops, guttation and surface water and from soil.

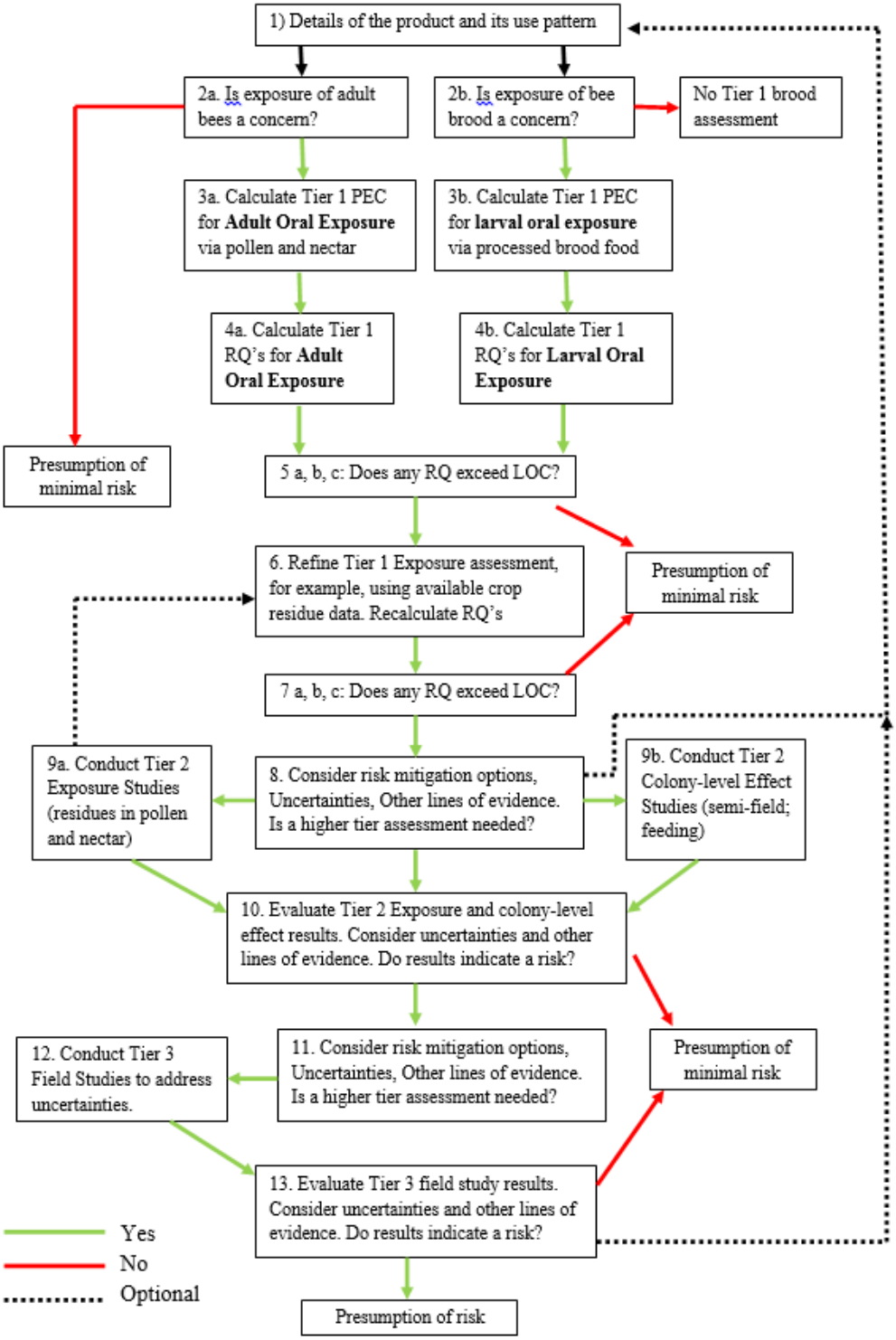
[14] USEPA, 2012. Label Review Manual. www.epa.gov/oppfead1/labeling/lrm/

APPENDIXES

APPENDIX A: PROPOSED TIERED APPROACH FOR ASSESSING RISK TO HONEY BEES FROM FOLIAR APPLICATION



APPENDIX B: PROPOSED TIERED APPROACH FOR ASSESSING RISK TO HONEY BEES FROM SOIL/SEED TREATMENTS



APPENDIX C: EFSA SCREENING LEVEL ASSESSMENT METHODOLOGY, HONEY BEES

The following appendix describes the EFSA screening risk assessment process where potential exposure is identified.

Foliar application

1. Calculate the hazard quotient (HQ) contact using the following:

   HQcontact = AR/LD50contact

   Where AR = application rate (g ac/ha); LD50 contact in µg ac/bee.

   EFSA (2013) accept a hazard quotient of ≤ 42 for application made *via* a downwards spray, or ≤ 85 for an application made *via* an upwards and/or sideward spray.

2. Calculate the acute exposure-toxicity (ETR) ratio for adults using the following:

   ETRacute adult oral = AR X SV/LD50oral

   Where AR = application rate (g ac/ha); SV = shortcut value; LC50 oral in µg ac/bee.

   Rationale behind the shortcut values are described in EFSA (2013) and not repeated here. They relate to residue levels and dietary intake. For an application made *via* a downwards spray, the SV = 7.55 for an application made *via* an upwards and/or sideward spray, the SV = 10.6.

   EFSA (2013) accept ETR of ≤ 0.2 for the acute assessment.

3. Calculate the **ETRchronic adult oral** using the following:

   ETRchronic adult oral = AR X SV/LC50oral

   Where AR = application rate (g ac/ha); SV = shortcut value; LC50 oral in µg ac/bee/day.

   The SV = 7.55 for an application made *via* an upwards and/or sideward spray, the SV = 10.6.

   EFSA (2013) accept ETR of ≤ 0.03 for the chronic assessment.

4. Calculate the **ETRlarvae** using the following:

   ETRlarvae = AR X SV/NOEClarvae

   Where AR = application rate (g ac/ha); SV = shortcut value; NOEC larvae in µg ac/larvae per developmental period.

   The SV = 4.4 for an application made *via* an upwards and/or sideward spray, the SV = 6.1.

   EFSA (2013) accept an ETR of ≤ 0.2 for this larvae assessment.

Exposure *via* pollen and nectar

1. Calculate the **ETRacute adult oral** using the following:

$$\text{ETRacute adult oral} = \text{AR X Ef X SV/LD50oral}$$

Where AR = application rate (g ac/ha); Ef = exposure factor; SV = shortcut value; LC50 oral in µg ac/bee.

Rationale behind the exposure factor (Appendix X; EFSA, 2013) and shortcut values (Appendix J; EFSA, 2013) are described in EFSA (2013) and not repeated here. Shortcut values are provided in Tables J4-J7, Appendix J EFSA (2013).

EFSA (2013) accept ETR of ≤ 0.2 for the acute assessment.

2. Calculate the **ETRchronic adult oral** using the following:

$$\text{ETRchronic adult oral} = \text{AR X Ef X SV X twa/LC50oral}$$

Where AR = application rate (g ac/ha); Ef = exposure factor; SV = shortcut value; twa = time weighted average; LC50 oral in µg ac/bee/day. The default twa is 0.72, which is based on a default DT50 of 10 days and a 10-day time window.

EFSA (2013) accept ETR of ≤ 0.03 for the chronic assessment.

3. Calculate the **ETRlarvae** using the following

$$\text{ETRlarvae} = \text{AR X Ef X SV X twa/NOEClarvae}$$

Where AR = application rate (g ac/ha); Ef = exposure factor; SV = shortcut value; twa = time weighted average; LC50 oral in µg ac/bee/day. The default twa is 0.85, which is based on a default DT50 of 10 days and a 5-day time window.

EFSA (2013) accept ETR of ≤ 0.2 for this larvae assessment.

Seed treatment or granules applied at drilling or incorporated into the soil

1. Calculate the hazard quotient (HQ) contact using the following:

$$\text{HQcontact} = \text{Fdep X AR/LD50contact}$$

Where Fdep = fraction of the dose deposited on the type of plants that foragers will visit (Appendix X of EFSA 2013); AR = application rate (g ac/ha); LD50 contact in µg ac/bee.

EFSA (2013) accept a hazard quotient of ≤ 14 for application made *via* a downwards spray.

2. Calculate the **ETRacute adult oral** using the following:

   i. ETRacute adult oral = AR X Ef X SV/LD50oral (**soil applied)**

Where AR = application rate (g ac/ha); Ef = exposure factor; SV = shortcut value; LC50 oral in µg ac/bee.

Rationale behind the exposure factor (Appendix X; EFSA, 2013) and shortcut values (Appendix J; EFSA, 2013) are described in EFSA (2013) and not repeated here. The SV is 7.55 and Ef is 0.3.

ii. ETRacute adult oral = AR X SV/LC50oral (**seed treatment)**

Where AR = application rate (mg ac/seed); SV = shortcut value (= 0.78); LD50 oral in µg ac/bee.

EFSA (2013) accept ETR of ≤ 0.2 for the acute assessment.

3. Calculate the **ETRchronic adult oral** using the following:

i. ETRchronic adult oral = AR X Ef X SV / LC50oral (**soil applied)**

Where AR = application rate (g ac/ha); Ef = exposure factor (=0.3); SV = shortcut value (=7.55); LC50 oral in µg ac/bee/day.

ii. ETRchronic adult oral = AR X SV / LC50oral (**seed treatment)**

Where AR = application rate (mg ac/seed); SV = shortcut value (=0.78); LC50 oral in µg ac/bee/day.

EFSA (2013) accept ETR of ≤ 0.03 for the chronic assessment.

4. Calculate the **ETRlarvae** using the following

i. ETRlarvae = AR X Ef X SV / NOEClarvae (**soil applied**)

Where AR = application rate (g ac/ha); Ef = exposure factor (=0.3); SV = shortcut value (=4.4); LC50 oral in µg ac/bee/day.

ii. ETRlarvae = AR X SV / NOEClarvae (**seed treatment)**

Where AR = application rate (mg ac/seed); SV = shortcut value (=0.4); LC50 oral in µg ac/bee/day

EFSA (2013) accept ETR of ≤ 0.2 for this larvae assessment.

Granules that are broadcast

1. Calculate the hazard quotient (HQ) contact using the following:

HQcontact = 0.1 X AR/LD50contact

Where AR = application rate (g ac/ha); LD50 contact in µg ac/bee.

EFSA (2013) accept a hazard quotient of ≤ 14.

2. Calculate the **ETRacute adult oral** using the following:

$$\text{ETRacute adult oral} = AR \times Ef \times SV / LC50\text{oral}$$

Where AR = application rate (g ac/ha); Ef = exposure factor (=0.3); SV = shortcut value (=7.55); LC50 oral in µg ac/bee.

EFSA (2013) accept ETR of ≤ 0.2 for the acute assessment.

3. Calculate the **ETRchronic adult oral** using the following:

$$\text{ETRchronic adult oral} = AR \times Ef \times SV / LC50\text{oral}$$

Where AR = application rate (g ac/ha); Ef = exposure factor (=0.3); SV = shortcut value (=7.55); LC50 oral in µg ac/bee/day.

EFSA (2013) accept ETR of ≤ 0.03 for the chronic assessment.

4. Calculate the **ETRlarvae** using the following

$$\text{ETRlarvae} = AR \times Ef \times SV / \text{NOEClarvae}$$ (**soil applied**)

Where AR = application rate (g ac/ha); Ef = exposure factor (=0.3); SV = shortcut value (=4.4); LC50 oral in µg ac/bee/day.

EFSA (2013) accept ETR of ≤ 0.2 for this larvae assessment.

GLOSSARY AND ABBREVIATIONS

ac	Active constituent
EEC	Estimated exposure concentration
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organisation
ETR	Exposure toxicity ratio
HQ	Hazard quotient
ICPBR	International Commission for Plant-Bee Relationship[1]
LC50	dose required to kill half the members of a tested population after a specified test duration
NOAEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted exposure concentration
RT25	Residual time to 25 per cent mortality (from foliage study designed to determine the length of time over which field weathered foliar residues remain toxic to honey bees)
RUD	Residue unit dose
SETAC	Society of Environmental Toxicology and Chemistry
US EPA	United States Environmental Protection Agency

1) In 2011 the name was amended to The International Commission for Plant-Pollinator Relationships (ICPPR).

REFERENCES

Alix A, Bakker F, Barrett K, Brühl C, Coulson M, Hoy S, Jansen J, Jepson P, Lewis G, Neumann P, Süβenback D & Van Vliet P, 2012. ESCORT 3. Linking non-Target Arthropod Testing and Risk Assessment with Protection Goals. Society of Environmental Toxicology and Chemistry (SETAC). ISBN 978-1-880611-98-2

Alix A, Chauzat MP, Duchard S, Lewis G, Maus C, Miles MJ, Pilling E, Thompson HM & Wallner K, 2009. Environmental Risk Assessment Scheme for Plant Protection Products—Chapter 10: Honey bees—Proposed Scheme. pp 27–33 in Oomen PA & Thompson HM (Editors) International Commission for Plant-Bee Relationships Bee Protection Group 10th International Symposium Hazards of Pesticides to Bees, Bucharest (Romania). October 8–10, 2008. Julius–Kühn Archiv

Aupinel P, Fortini D, Dufour H, Taseï JN, Michaud B, Odoux JF & Pham-Delègue MH, 2005. Improvement of artificial feeding in a standard *in vitro* method for rearing *Apis mellifera* larvae. Bull Insectol 58: pp 107–11

Babendreier D, Kalberer N, Romeis J, Fluri P & Bigler F, 2004. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. Apidologie 35: pp 293–300

Crailsheim K, Schneider LHW, Hrassnigg N, Bühlmann G, Brosch U, Gmeinbauer R & Schöffmann B, 1992. Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function. J. Insect Physiol 38 (6): pp 409–19

Crailsheim K, Hrassnigg N, Gmeinbauer R, Szolderits MJ, Schneider LHW & Brosch U, 1993. Pollen utilization in non-breeding honeybees in winter. J. Insect Physiol 39 (5): pp 36–373

Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S & Pham-Delgue MH, 2005. Comparative Sub-lethal Toxicity of Nine Pesticides on Olfactory Learning Performances of the Honeybee *Apis mellifera*. Arch. Environ Contam Toxicol 48: pp 242–50

EFSA, 2012. Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera, Bombus* spp. and solitary bees). European Food Safety Authority. EFSA Journal 2012; 10(5):2668f

EFSA, 2013. Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). European Food Safety Authority. EFSA Journal 11(7): pp 3295

EPPO, 2010a. Environmental risk assessment scheme for plant protection products, Chapter 10. Risk assessment to honey bees, PP 3/10 (3), OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 40: pp 1–9

EPPO, 2010b. EPPO standards PP1/170-Test methods for evaluating the side effects of plant protection products on honeybees. Bull OEPP/EPPO Bull 31: pp 323–30

Fischer D & Moriarty T, 2011. Pesticide risk assessment for pollinators: Summary of a SETAC Pellston Workshop. Pensacola FL (USA): Society of Environmental Toxicology and Chemistry (SETAC).

Girolami V, 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): pp 1808–15

Luttik R, Hart A, Roelofs, W, Craig P & Mineau P, 2011. Variation in the level of protection afforded to birds and crustaceans exposed to different pesticides under standard risk assessment procedures. Integrated environmental assessment and management, 7: pp 459–65

Mensink BJWG, Montforts M, Wijkhuisen-Maślankiewicz L, Tibosch H & Linders JBHJ, 1995. Manual for summarising and evaluating the environmental aspects of pesticides, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands, Report no. 67910122, July 1995

OECD 1998a. OECD Guidelines for the testing of chemicals, Number 213. Honeybees, acute oral toxicity test, OECD, Environmental Health and Safety Division, Paris

OECD 1998b. Guidelines for the testing of chemicals, Number 214. Honeybees, acute contact toxicity test, OECD, Environmental Health and Safety Division, Paris

OECD, 2007. Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Series on Testing and Assessment Number 75. Guidance Document on the Honey Bee (*Apis mellifera* L.) Brood Test under Semi-Field Conditions. ENV/JM/MONO(2007)22. Organisation for Economic Co-operation and Development (OECD), 31 August 2007

OECD, 2013. Guideline for the testing of Chemicals No 237. Honey bee (*Apis mellifera*) larval toxicity test, single exposure OECD, Paris. 26 July 2013. Available at: www.oecd-ilibrary.org/docserver/download/9713171e.pdf?expires=1378944843&id=id&accname=guest&checksum=7B02767226D890D552BCD7E2EA2F2B81

Pistorius J, 2014. From hazard to risk: Dusts, guttation, and other routes of exposure and the risk to pollinators. 13th IUPAC International Congress of Pesticide Chemistry, August 10–14, 2014, San Francisco, California, USA Abstract AGRO 705, p 183

Oomen PA, De Ruijter A & Van der Steen J, 1992. Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bull OEPP/EPPO Bull 22: pp 613–16

Rortais A, Arnold G, Halm MP & Touffet-Briens F, 2005. Modes of honey bees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36: pp 71–83

SCEW, 2009. Environmental Risk Assessment Guidance Manual for Agricultural and Veterinary Chemicals. Australian Standing Council on Environment and Water. www.scew.gov.au/resource/chemical-risk-assessment-guidance-manuals

Schur A, Tornier I, Brasse D, Mühlen W, Von der Ohe W, Wallner K & Wehling M, 2003. Honey bee brood ring-test in 2002: method for the assessment of side effects of plant protection products on the honey bee brood under semi-field conditions, Bulletin Insectol 56(1): pp 91–96

Simpson, J. 1955. The significance of the presence of pollen in the food of worker larvae of the honey bee. Quarterly Journal of Microscopical Science, 96(1): pp 117–20

US EPA, 1996a. OPPTS 850.3020: Honey Bee Acute Contact Toxicity; EPA 712-C-96-147; April 1996

US EPA, 1996b. OPPTS 850.3040: Field Pollinator Studies; EPA 712-C-96-150; April 1996

US EPA, 2010. Weight of Evidence Guidance: Evaluating Results of EDSP Tier-1 Screening to Identify Candidate Chemicals for Tier-2 Testing. Draft for Public Comment. www.epa.gov/endo/pubs/EDSP_Draft_WoE_Paper_%20for_Public_Comments%20_2010.pdf

US EPA, 2011. Interim Guidance on Honey Bee Data Requirements. Environmental Fate and Effects Division, Office of Pesticide Programs. 19 October 2011

US EPA, 2012a. Ecological Effects Test Guidelines. OCSPP 850.3030. Honey bee toxicity of residues on foliage. EPA 712-C-018. January 2012

US EPA, 2012b. Label Review Manual. Chapter 8: Environmental Hazards. Available at: www.epa.gov/oppfead1/labeling/lrm/

US EPA, Health Canada PMRA and California Department of Pesticide Regulation, 2012. White Paper in Support of the Proposed Risk Assessment Process for Bees. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs. Environmental Fate and Effects Division; Pest Management Regulatory Agency, Health Canada. 11 September 2012

US EPA, Health Canada PMRA and California Department of Pesticide Regulation, 2014. Guidance for Assessing Pesticide Risks to Bees. 19 June 2014. Available at: www2.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf

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