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<th>Description</th>
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<td>ACPPO</td>
<td>Australian Chief Plant Protection Office</td>
</tr>
<tr>
<td>AHA</td>
<td>Animal Health Australia</td>
</tr>
<tr>
<td>AHBIC</td>
<td>Australian Honey Bee Industry Council</td>
</tr>
<tr>
<td>APVMA</td>
<td>Australian Pesticides and Veterinary Medicines Authority</td>
</tr>
<tr>
<td>CCEPP</td>
<td>Consultative Committee on Emergency Plant Pests</td>
</tr>
<tr>
<td>CPHM</td>
<td>Chief Plant Health Manager</td>
</tr>
<tr>
<td>DEPI</td>
<td>Department of Environment and Primary Industries</td>
</tr>
<tr>
<td>DAFWA</td>
<td>Department of Agriculture and Food Western Australia</td>
</tr>
<tr>
<td>DPIF</td>
<td>Department of Primary Industry and Fisheries</td>
</tr>
<tr>
<td>DPIPWE</td>
<td>Department of Primary Industries, Parks, Water and Environment</td>
</tr>
<tr>
<td>HAL</td>
<td>Horticulture Australia Ltd</td>
</tr>
<tr>
<td>MRL</td>
<td>Maximum Residue Limit</td>
</tr>
<tr>
<td>NAQS</td>
<td>Northern Australian Quarantine Strategy</td>
</tr>
<tr>
<td>NBPSP</td>
<td>National Bee Pest Surveillance Program</td>
</tr>
<tr>
<td>NSW DPI</td>
<td>New South Wales Department of Primary Industries</td>
</tr>
<tr>
<td>OIE</td>
<td>World Organisation for Animal Health</td>
</tr>
<tr>
<td>PHA</td>
<td>Plant Health Australia</td>
</tr>
<tr>
<td>PIRSA</td>
<td>Primary Industries and Regions South Australia</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>QDAFF</td>
<td>Queensland Department of Agriculture, Fisheries and Forestry</td>
</tr>
<tr>
<td>RIRDC</td>
<td>Rural Industries Research and Development Corporation</td>
</tr>
<tr>
<td>SHB</td>
<td>Small hive beetle</td>
</tr>
<tr>
<td>TBA</td>
<td>Tasmanian Beekeepers Association</td>
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PHA would also like to thank everyone who provided comments and reviewed this Operations Manual.
1 Introduction

The National Bee Pest Surveillance Program (NBPSP) is an early warning system to detect new incursions of exotic bee pests and pest bees. The Program involves a range of surveillance methods conducted at locations considered to be of most likely entry of bee pests and pest bees throughout Australia. Early detection of these pests is critical to providing the best possible opportunity to eradicate an incursion, and to limiting the size and cost of an eradication program. The program also contributes to competitive market access for Australia’s queen bees and packaged bees.

The NBPSP is jointly funded by the Australian Honey Bee Industry Council (AHBIC), Horticulture Australia Ltd (HAL), Australian Government through the Department of Agriculture and the Rural Industries Research and Development Corporation (RIRDC). In-kind contributions for the implementation of the program are provided through each State and Territory Department of Agriculture. At a national level, Plant Health Australia (PHA) coordinates and administers the Program.

For the purposes of the NBPSP, the term, ‘surveillance’ is used in a general sense to include the activities of monitoring and surveillance as defined in the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE).

2 Background

The NBPSP follows on from the National Sentinel Hive Program which was established in 2000 to improve post-border monitoring around Australia for exotic pests of honey bees. In January 2012 the management of the National Sentinel Hive Program was transferred from Animal Health Australia (AHA) to PHA. This followed the transfer in responsibilities for bees at a national level from animal biosecurity to plant biosecurity. These transfers did not greatly change the national implementation of the Program, which is delivered through the expertise of state and territory apiary officers and volunteer beekeepers.

However, upon the transfer to PHA the name of the surveillance Program was changed to the NBPSP to reflect a transition to a more broadly based surveillance Program for bee pests and pest bees.

Seagoing vessels are considered to present a significant risk for the transportation to Australia of exotic bees (and associated parasites) either in superstructure, containers or equipment, or in vessel holds. The Asian honey bee (Apis cerana), Giant honey bee (Apis dorsata) and Africanised honey bee (Apis mellifera scutellata) have all been detected and intercepted on ships destined for Australia or in port areas in recent years. The incursion and establishment of the Asian honey bee (Apis cerana Java genotype) in the Cairns region from 2007 onwards also confirmed the risk of incursions by exotic honeybee pests via ocean-going vessels that enter Australian ports.

The NBPSP is currently primarily based on sentinel hives, which are hives of European honey bees (Apis mellifera) of a known health status. As of May 2014, there are around 130 hives maintained at 29 air and sea ports around Australia that receive a significant
volume of cargo and are believed to be of high risk (see Appendix 1). The NBPSP attempts to have at least six hives deployed within three km of the highest risk ports in each jurisdiction. The sentinel hives are provided, managed and tested by cooperating beekeepers under the support of AHBIC, or in some cases, the hives are provided, managed and tested by the respective State/Territory Department of Agriculture.

Hives are tested every two months using an acaricide (miticide) to provide a means of early detection of Varroa mites (*Varroa destructor* and *V. jacobsoni*) and Tropilaelaps mites (*Tropilaelaps clareae* and *T. mercedesae*), which could potentially enter via exotic bees on a vessel or transported cargo. Samples of bees are also taken from these sentinel hives every two months and submitted for dissection and examination for Tracheal mite (*Acarapis woodi*), which also could enter via exotic bees. From 2013 onwards, additional surveillance techniques such as sugar shaking, alcohol washing and drone uncapping have also been included for the detection of exotic bee pests at additional high risk locations.

To detect the possible incursion of pest bees, surveillance techniques such as catch boxes (empty hives) and swarm capture of bees at ports are conducted. In addition, remote sensing of beehives (catchboxes with cameras) and floral sweep netting will also be included for the early detection of exotic species of Asian honey bee (*Apis cerana*), Giant honey bee (*A. dorsata*), Red dwarf honey bee (*A. florea*) or Bumblebees (*Bombus* spp).

In June 2013, surveillance for Small hive beetle (*Aethina tumida*) was incorporated into the Program for Tasmania and the Northern Territory, where it is currently not present, and also Western Australia, where it is currently restricted in distribution to the northern part of the state (Kununurra). Hives are tested every two months using oil traps or Apithor harbourages (containing the insecticide fipronil). This routine testing provides a means for early detection of Small hive beetle (SHB) as well as supporting export market access for Tasmania, the Northern Territory and parts of Western Australia through the collection of data demonstrating pest absence.

### 3 Purpose

The purpose of the NBPSP is to provide information on Australia’s honey bee industry health status to support the beekeeping and horticultural industries, facilitate trade in honey bee industry commodities and meet Australia’s international reporting obligations. It also provides information on Australia’s capabilities and activities with regard to surveillance and control of honey bee pests and pest bees.

The NBPSP and this Operations Manual are not meant to provide an analysis of all bee biosecurity and surveillance work that is conducted at the pre-border, border and post-border, or detail specific and additional surveillance activities that may be conducted by specific agencies or government departments.
4 Objectives

The NBPSP supports the following objectives:

1. **To provide an early warning system for exotic bee pests and pest bees:** The NBPSP acts as an early warning system for the detection of new incursions of exotic bee pests and pest bees. This greatly increases the possibility of eradicating an incursion, and limits the scale and cost of an eradication program.

2. **To provide trade support:** The NBPSP facilitates the export of queen bees and packaged bees to countries sensitive to a range of bee pests and pest bees. The Program provides technical, evidence based information to support Australia’s pest free status claims during export negotiations and will assist exporters in meeting export certification requirements.

5 Outputs

All data is recorded in the NBPSP online interface [http://nbpsp.planthealthaustralia.com.au](http://nbpsp.planthealthaustralia.com.au). PHA as administrators of the program are the only agency with access to this database.

All data on a yearly basis is reported in the *Animal Health in Australia* report, prepared by the Department of Agriculture annually and presented at the World OIE meeting, as well as the *National Plant Biosecurity Status Report*, prepared by PHA annually and distributed to stakeholders. These are the only formal reporting avenues currently available; however, requests for summary reports can be received at any time.

6 Roles

The efficient and effective operation of the NBPSP is dependent upon the contribution of various individuals fulfilling defined roles within the program. These roles include the National Coordinator and Industry or Jurisdictional Coordinators. The role descriptions and responsibilities are detailed below. The names and contact details of staff currently filling these roles are listed in Table 1, page 14.

6.1 National Coordinator

The role of the National Coordinator is to coordinate the planning and implementation of NBPSP activities across Australia to ensure that the NBPSP objectives are met. The position is currently held by PHA, with support provided by the Department of Agriculture.

The primary responsibilities of PHA include:

- National coordinator and administrative contact point.
- Writing program reports.
• Purchasing sticky mats, chemical strips and Apithor traps on behalf of the program and mailing these out to state/territory coordinators.
• Data entry on behalf of state/territory coordinators.
• Finalising contracts with all parties involved.
• Maintenance of APVMA permits.
• Maintenance of the Operations Manual.

The primary responsibilities of the Department of Agriculture include:

• Acting as the primary permit holder for pesticides used in the Program.
• Involvement and assistance in conducting surveillance activities at designated high risk ports
• Auditing of chemicals used within sentinel hives.
• Coordinating emergency response arrangements in the event of an incursion.

PHA’s role as coordinator is funded from the Program, which is contributed to by AHBIC, HAL and the Commonwealth Department of Agriculture.

6.2 Jurisdictional Coordinators

The role of jurisdictional coordinators is to manage and coordinate NPBSP activities within their own state/territory to ensure that the NPBSP objectives are met. In New South Wales, the Northern Territory, Queensland, South Australia, Victoria and Western Australia the State/Territory Department of Primary Industries take the lead role in coordinating surveillance activities.

Although the amount and type of surveillance activities conducted and coordinated by jurisdictional coordinators can vary between each state or territory, jurisdictional coordinators are responsible for the overarching activities of the NBPSP. This includes:

• That all aspects of the contract (and attachments) between the jurisdiction and PHA are completed on time and at a high standard.
• Ensuring that testing is completed every 2 months and that completed data forms are forwarded to PHA within 2 weeks of completion.
• Maintaining a list detailing the location of all sentinel hives, and the detail of who manages the sentinel hives. This information will be requested at the start of the financial year to be reviewed and/or updated.
• Ensuring storage, usage and disposal of chemical (Bayvarol or Apistan) strips is undertaken according to the label (i.e. ensuring control over all chemical strips is maintained until their appropriate disposal – such that all strips and their use is able to be accounted for).
• Reporting any suspect detection/s of exotic mite/s to the Chief Plant Health Manager in their jurisdiction within 24 hours and dispatching material for confirmatory diagnostics.
• Negotiating with port authorities and other landholders for the placement of sentinel hives.
• Provision of information to volunteer beekeepers used within the program on sample assessment and workplace health and safety procedures.
• Ensuring the integrity of the sentinel hives, catchboxes and any other surveillance tools that are used.

In Tasmania, the lead role of jurisdictional coordinator is taken by industry, through the Tasmanian Beekeepers Association (TBA). TBA manages and administers the Tasmanian component of the NBPSP through liaising and coordinating beekeepers who manage sentinel hives. Surveillance activities are coordinated with the Department of Primary Industries, Parks, Water and Environment (DPIPWE) who are responsible for providing diagnostic support for the Tasmanian component of the Program through the Plant Biosecurity and Diagnostics Branch of DPIPWE.

The primary responsibilities of the TBA are the same as those listed above for other jurisdictions, with a few minor differences:

• Maintaining a list detailing the location of all sentinel hives, and the detail of who manages the sentinel hives. This information will be requested at the start of the financial year to be reviewed and/or updated.
• Ensuring storage, usage and disposal of chemical (Bayvarol or Apistan) strips is undertaken according to the label (i.e. ensuring control over all chemical strips is maintained until their appropriate disposal – such that all strips and their use is able to be accounted for).
• Provision of information to volunteer beekeepers used within the program on sample assessment and workplace health and safety procedures.
• Ensuring the integrity of the sentinel hives, catchboxes and any other surveillance tools that are used.
• Ensuring that testing is completed every 2 months and that sticky mats and bee samples are forwarded to DPIPWE for diagnostics.
• The responsibility for reporting suspect exotic mite/s lies with DPIPWE since their role is to conduct the diagnostics (see below).

The primary responsibilities of DPIPWE include:

• Liaising with TBA to ensure that testing coordinated by the TBA beekeepers is on schedule.
• Ensuring that testing is completed every 2 months and that completed data forms are forwarded to PHA within 2 weeks of completion.
• Assessing sticky mats for external mites (Varroa sp. and Tropilaelaps sp.) and submitting results to PHA (including nil results).
• Assessing samples of bees collected for Tracheal mite (Acarapis woodi) and submitting results to PHA (including nil results).
• Reporting any suspect detection/s of exotic mite/s to the Chief Plant Health Manager in their jurisdiction within 24 hours and dispatching material for confirmatory diagnostics.
### Table 1. Jurisdictional coordinator contacts

<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Contact details</th>
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<tbody>
<tr>
<td><strong>NSW Department of Primary Industries (NSW DPI)</strong></td>
<td></td>
<td></td>
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<td>NSW DPI</td>
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<tr>
<td></td>
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<td>Mobile: 0427 311 410</td>
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<td>(PO BOX 389)</td>
<td>Fax: (02) 4822 3261</td>
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<tr>
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<td>Email: <a href="mailto:doug.somerville@dpi.nsw.gov.au">doug.somerville@dpi.nsw.gov.au</a></td>
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<tr>
<td>Karen Webster</td>
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</tr>
<tr>
<td></td>
<td>159 Auburn St</td>
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<td></td>
<td>(PO BOX 389)</td>
<td>Email: <a href="mailto:karen.webster@dpi.nsw.gov.au">karen.webster@dpi.nsw.gov.au</a></td>
</tr>
<tr>
<td></td>
<td>Goulburn NSW 2580</td>
<td></td>
</tr>
<tr>
<td><strong>Department of Primary Industry and Fisheries (NT DPIF)</strong></td>
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7 Methodology for surveillance and diagnostic techniques used in the NBPSP

This section covers the methodology for the surveillance and diagnostic techniques used in the NBPSP. These methods support early detection of exotic mites (Varroa, Tropilaelaps and Tracheal mites), and honey bees (Giant, Red dwarf, Africanized and Cape honey bees) as well as SHB in Northern Territory, Tasmania and southern Western Australia (where it is absent) and new incursions of Asian honey bee, as well as the extension of range of the existing Asian honey bee population in Cairns, Qld. Preliminary diagnostic information on these pests is also provided, however this is not intended to be a formal diagnostic manual. Following preliminary examination, suspect exotic pest samples should be forwarded to the laboratory in the relevant state/territory for confirmatory diagnostics.

Current methods of exotic mite surveillance are primarily based on sentinel hives. These hives work on the principle that testing the local population of honey bees around a high risk port area will provide an early warning system for the entry of exotic mites. Varroa mites may be transferred to and from honey bees visiting the same flower (Kevan et al., 1990) and can therefore spread via foraging or drifting honey bees. They can also attach themselves to other flower-visiting insects such as Bombus spp., flies (Syrphidae), beetles (Scarabaeidae) and wasps (Vespidae). Therefore, if honey bees carrying exotic mites enter the country via vessels or cargo at high risk ports, they could transfer the mites to the local A. mellifera population when foraging and then be picked up through sentinel hive surveillance. The presence/absence of mites is assessed every 2 months through the use of acaricide strips and sticky mats. The acaricide kills the mites present in the hive, which drop onto a sticky mat that can then be inspected.

Other methods for detection of exotic mites covered in this manual include sugar shaking, alcohol washing and drone uncapping. These methods can be supplemented with community (i.e. hobby beekeeper) involvement in surveillance activities (e.g. sugar shaking and alcohol washing) in and around the high risk port areas. In addition, swarms and/or feral nests captured from in and around high risk port areas (including from catchboxes/remote catchboxes) can provide early detection of exotic mites which may be carried on exotic bees or newly arriving European honey bee or Asian honey bee swarms.

Sentinel hives can also be used to provide early detection of SHB in states/territories where it is not currently present. Methodology for the use of beetle traps (e.g. Apithor and oil) in hives is described for detection of SHB.

Surveillance methods for the detection of exotic bees covered in this manual include the capture of swarms and/or feral nests detected in and around high risk port areas or from catchboxes or remote catchboxes, in addition to floral sweep netting and collection of rainbow bee-eater pellets.
7.1 Surveillance methods for detection of Varroa mites

Surveillance methods for Varroa mites (Varroa destructor, V. jacobsoni) include the use of Bayvarol or Apistan acaricide strips and sticky mats in sentinel hives, sugar shaking and alcohol washing of bees, drone uncapping and examination of swarms captured in and around high risk port areas (including from catchboxes/remote catchboxes), for the presence of exotic mites. These methods can be supplemented with community (i.e. hobby beekeeper) involvement in surveillance activities (e.g. sugar shaking and alcohol washing) in and around the high risk port areas.

7.1.1 Sentinel hives

Sentinel hives should be placed as close to the port of entry as possible (at least within 3 km) at a density of at least 6 hives per port (Barry et al 2010). The use of an 8 frame hive is preferable. Where 8 frame hives are unavailable, 10 frame hives may be used, however, floor coverage by sticky mats will not be as good. Ensure the bottom board is separate from the brood box. The support riser on the bottom board at the opposite end to the hive entrance should be removable without lifting the brood box from the bottom board. This allows the holder with the sticky mat and its wire gauze to be placed in the base of the hive. The wire gauze cover prevents bees getting stuck but allows mites to fall through.

7.1.1.1 Inserting acaricide strips and sticky mats into hives

Assessment of sentinel hives using Bayvarol or Apistan acaricide strips and sticky mats is undertaken every 8 weeks. The procedure for inserting strips and sticky mats is described below. Strips must be used in accordance with the APVMA minor use permit requirements (see Appendix 3, page 108 for a copy of the permit). Product labels and MSDS should be read prior to use (see Appendix 5, page 118 for a copy of product labels and MSDS).

1. To insert the sticky mat, first remove the gauze from the sticky mat holder.
2. Peel the paper from the sticky mat to reveal the sticky surface. Keep this paper because it will be put back on the sticky mat after its removal from the hive and prior to sending away for analysis.
3. Lay the sticky mat, sticky side up, on the holder.
4. Place the wire gauze over the mat.
5. Apply some smoke to the hive and take out the removable support riser at the rear of the hive.
6. Slide the sticky mat holder in along the floor of the hive. It should be designed so that the mat is in the centre of the hive. Make sure it is fully pushed in. This will be achieved when the rear of the hive is sealed.
7. Use only new Bayvarol or Apistan strips when treating the hive. Use the number of strips as specified on the label (see page 108) – Please note that strips are to be
used only once and then disposed of in a manner according to the label. This information (date of placement, removal and disposal of strips) must be recorded.

8. Strips should be placed between frames within the brood box with the lugs at the top. Strips should be separated by two or three frames in the middle of the box. Hang the strips on the top bars of the frames.

9. Ensure strips are hung over the sticky mats below.

10. Leave the strips in the hive for 48 hrs (Note - this time period may differ from the Bayvarol label example supplied but complies with permit 14167).

11. After 48 hrs, remove the strips and dispose of them according to the label and record this information.

12. Remove the sticky mat and holder and return the hive back to normal.

13. Remove the sticky mat from the holder and replace the paper cover onto the sticky mat.

14. The sticky mat and paper cover are rolled up, sticky side in and placed into a postal tube if to be sent for diagnosis.

7.1.1.2 Examination of sticky mats

1. To examine the sticky mat for suspect mites, cut into strips and if possible examine under a dissecting microscope. If necessary or desired, specimens can be removed from the sticky mat and mounted prior to viewing under the microscope (follow steps 3 - 5).

2. Identify and count any invertebrates found.

3. If necessary, remove specimens from sticky mat by cutting out the relevant section of mat and soaking in citrus oil solvent (e.g. Citroclean or Histolene) until specimen is detached and clean.

4. Specimens can then be soaked in 70% ethanol and mounted appropriately.

5. Varroa and Tropilaelaps mites are heavily sclerotised and do not require staining. Mount on microslides for viewing. Larger arthropods such as beetles and Braula fly should be pinned for viewing and stored in 70% ethanol.

6. Suspect exotic pests found following sticky mat inspection should be examined closely to determine if they are exotic. Refer to Preliminary identification of suspected exotic mites (page 33) for images of Varroa mites, Tropilaelaps mites, Braula fly, SHB and common external mites they could be confused with.

7. Record the presence or absence of Varroa mites, Tropilaelaps mites and Braula fly in the excel workbook (Data capture form).

8. If a suspected exotic mite (or Braula fly) is found and is still attached to the sticky mat, cut out the relevant section of sticky mat and place in a labelled press seal (zip-lock) bag. Freeze for 24 hrs to ensure mites are killed. Alternatively, soak the section of sticky mat in citrus oil solvent (e.g. Citroclean or Histolene) until specimen is detached from the sticky board and clean and place specimen in a plastic or glass vial filled with 80% ethyl alcohol e.g. methylated spirits).
9. Contact your local department of agriculture entomologist to arrange diagnostics and to determine the preferred method of specimen preparation/preservation prior to sending samples.

10. In the event a suspected exotic mite or insect is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

11. It has been reported that Varroa mites may be carried on people (D Anderson, CSIRO Entomology, pers comm, November 2004). Therefore, if Varroa mites are detected, decontamination of clothes and body (including hair and beards if exposed) should be carried out.

### 7.1.1.3 MRL testing of honey and wax

As per condition 4 of APVMA Permit 14167 (see page 108), any honey from sentinel hives intended to be consumed, sold or supplied, must be tested according to the Maximum residue limits (MRL) for Flumethrin (Bayvarol) and Tau-Fluvalinate (Apistan) (see Maintenance of APVMA permits, page 99). Testing of the honey for these chemicals takes place every financial year during one of the surveillance periods (as negotiated with each state/territory).

Each jurisdiction can choose whether to send samples from each sentinel hive used, or to pool samples from specific locations for MRL testing (i.e. if 6 sentinel hives are contained in the one location, the honey from these hives can be pooled and then 1 sample sent away for analysis that reflects all hives MRL status in that location).

Honey samples (≥50g each) are to be sent from each hive/location tested to Agri-Solutions (address below). This testing is compulsory and is at the expense of each jurisdiction.

Each sample will roughly cost $319. Before sending off samples, email Andrew Keats, Manager at Agri-Solutions ([andrew.keats@agrisolutions.com.au](mailto:andrew.keats@agrisolutions.com.au)) and PHA and list:

- sentinel hive ID / location information
- the date you are posting the samples
- quote the consignment number
- courier company
- which chemical (Flumethrin – Bayvarol / or / Tau-fluvalinate - Apistan) you need to test for.

The postal address for samples is:
Attn. Scott Winner
AgriSolutions Australia
Unit 8, 2-8 Kabi Circuit
Deception Bay, QLD 4508
7.1.2 Sugar shaking

Sugar shaking of honey bees is a quick and easy method to detect Varroa mites. This method does not kill the bees and removes 70-90% of external Varroa mites present on adult honey bees. It should be conducted on at least 10% of hives in an apiary. The method works by the fine sugar particles dislodging Varroa mites by stopping their sticky pads (feet) gripping onto honey bees and also by stimulating grooming behaviour of honey bees. The sugar is then separated from the bees and inspected for mites. Note that this method will not detect very low infestations of Varroa mites in hives.

A designed version of this fact sheet is available at [www.beeaware.org.au/surveillancemethods](http://www.beeaware.org.au/surveillancemethods). This may be useful for your own use, or to hand out to assisting hobby beekeepers.

7.1.2.1 Equipment required

- Jar (preferably plastic) about 500 – 750 grams in size and lid with holes 3-5 mm in size (drilled or use 3mm gauze wire mesh)
- Pure icing sugar
- Cup (about 250 mL)
- Tablespoon
- Newspaper or large plastic sheet
- Container to hold water (ice cream container or small white bucket) or white sheet of paper/cardboard
- Protective clothing, smoker and hive tool
- Magnifying lens (if available)
- Filter paper (e.g. coffee filter) or fine sieve (optional)

7.1.2.2 Procedure

1. If using a container or bucket to collect sugar, half fill with water before commencing the sugar shake.
2. Place 1 tablespoon of icing sugar into the jar.
3. Place a large sheet of newspaper or plastic beside the hive to be tested.
4. Light a smoker, open the hive and remove a frame from near the centre of the brood. If possible, take adult bees from at least 3 brood frames. If the queen is present place her back in the hive.
5. Shake the bees off the frame onto the newspaper/plastic sheet and pour about 300 bees (1/2 a cup) into the jar (see Figure 1).
6. Put the lid on the jar quickly to prevent the bees from escaping.
7. Roll and gently shake the jar for 2-3 minutes, ensuring the honey bees are covered in sugar. Be careful not to lose any sugar. Do the shaking in a sheltered position protected from wind, so any mites present do not blow away.
8. Leave for 2-3 minutes before rolling and shaking again for another 2-3 minutes. The longer the bees are rolled in the sugar, the more effective the technique.
9. Shake the sugar out of the jar through the holes/mesh into a container/bucket half filled with water or shake onto a white sheet of paper/cardboard.

10. Release the bees from the jar onto the ground at the hive entrance in case queen is present (see Figure 2) and inspect the empty jar thoroughly for mites.

11. If the sugar was shaken into a bucket or container of water then the sugar will dissolve and any Varroa mites will float on the surface. Inspect the surface thoroughly for mites. A magnifying lens can be used if available.

12. Alternatively the water can be gently stirred to dissolve all the sugar and then passed through filter paper (e.g. coffee filter) which can then be thoroughly inspected for Varroa mites.

13. If the sugar was shaken onto a white sheet of paper or cardboard, the sugar needs to be spread finely across the paper to ensure any Varroa mites that are present are not covered with sugar particles. Inspect thoroughly for Varroa mites (see Figure 3).

14. Alternatively the sugar can be poured through a very fine sieve that will capture the Varroa mites while allowing the sugar to pass through. The sieve contents can then be thoroughly inspected on a sheet of white paper. Note that wind can be a major problem for this particular technique.

15. To assist with identification, see “Preliminary identification of suspected exotic mites”, page 33 for descriptions and images of exotic and other common mites.

16. Record the presence or absence of exotic mites in the excel workbook (Data capture form).

17. If exotic mites are suspected, place all specimens in a labelled plastic or glass vial and fill with 80% ethyl alcohol (e.g. methylated spirits), or freeze (in vial or zip-lock bag) for 24 hrs to ensure mites are killed. Contact your local department of agriculture entomologist to arrange diagnostics and to determine the preferred method of specimen preparation/preservation prior to sending samples.

18. Report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

19. It has been reported that Varroa mites may be carried on people (D Anderson, CSIRO Entomology, pers comm, November 2004). Therefore, if Varroa mites are detected, decontamination of clothes and body (including hair and beards if exposed) should be carried out.
Figure 1. Honey bees are poured into a jar for sugar shaking. Source: Ron Aggs, NSW DPI.

Figure 2. Honey bees coated in icing sugar are returned to the hive entrance after sugar shaking. Source: Randy Oliver, www.Scientificbeekeeping.com
7.1.3 Alcohol washing

Alcohol washing is a quick and effectively method for detecting the presence of Varroa mites, as well as monitoring colony mite levels. The disadvantage of this method is that it kills the bees that are sampled. The alcohol wash method can remove 70-80% of external Varroa mites present on adult honey bees. This technique is more effective when little brood is present, however it will provide measurable results when there is also significant quantities of brood and the sample bees are taken from the centre of the brood nest.

A designed version of this fact sheet is available at www.beeaware.org.au/surveillancemethods. This may be useful for your own use, or to hand out to assisting hobby beekeepers.

7.1.3.1 Equipment required

- 2 plastic jars, about 500 grams in size and with wide mouth
- 3 mm gauze wire mesh
- Soldering gun
- Cup (about 250 mL)
- 100 mL of 25% rubbing alcohol or 25% methylated spirits
- Newspaper or large plastic sheet
- Protective clothing, smoker and hive tool
- Magnifying lens (if available)
- A container (e.g. small white bucket)
- Filter paper (e.g. cloth, coffee filter)

7.1.3.2 How to make an alcohol washing kit

- Acquire two identical plastic jars (such as large peanut butter jars) with screw top lids.
• Carefully cut out the inner section of the closed-end of the screw-top lids.
• Cut out a 3 mm gauze wire mesh and place between the two open screw-top lids (Figure 4).
• Solder both lids together with the mesh in-between. A deep weld is required as a light weld may crack with use (Figure 5). Ensure that you are in a well-ventilated room when welding plastics due to toxic vapours that could be released from this process. (Please note: Plastic jar lids are commonly made from polypropylene and cannot be glued effectively. Therefore, you will need to heat weld them together.)
• Connect both of the jars to the lids and make sure that the lid connection is strong.
• You now have a functional mite shaker for conducting the alcohol washing test (Figure 6).

Figure 4. Plastic lid with the inner removed and covered with a section of 3mm wire mesh. Source: Randy Oliver, www.scientificbeekeeping.com

Figure 5. A soldering gun joining the two plastic lids together, with both inner lids removed and the 3mm wire mesh sandwiched between the lids. Source: Randy Oliver, www.scientificbeekeeping.com
7.1.3.3 Alcohol washing procedure 1

1. Place about 100 mL of alcohol (25%) in one of the jars, or enough so that the bees will be covered.
2. Place a large sheet of newspaper or plastic beside the hive to be tested.
3. Light a smoker, open the hive and remove a frame which contains a lot of brood. If the queen is present place her back in the hive.
4. Shake bees from a brood frame onto the newspaper/plastic sheet/plastic tub (Figure 7).
5. The field bees will mostly fly out of the tub immediately, leaving behind predominantly nurse bees which are likely to carry a greater quantity of phoretic Varroa mites.
6. Pour about 300 bees (1/2 a cup) into the jar containing around 100 mL of 25% rubbing alcohol or methylated spirits (Figure 8).
7. Put the solid lid on the jar quickly to prevent the bees from escaping (Figure 9).
8. Invert the shaker so that the bees are now in the top jar.
9. Shake the jar vigorously for 20 seconds, ensuring the honey bees are covered in alcohol. It is essential to maintain a vigorous shaking motion in order for the alcohol to swirl with the bees in the top jar (Figure 10).
10. After 20 seconds and the last shake, jiggle the jar so that the alcohol drains through the bees into the bottom jar. If you don’t jiggle, some of the mites may get stuck on the bees in the top jar.
11. Once settled, raise the bottom of the jar to view any mites that have been dislodged from the bees (Figure 11).
12. To assist with identification, see “Preliminary identification of suspected exotic mites”, page 33 for descriptions and images of exotic and other common mites.
13. Record the presence or absence of mites in the excel workbook (Data capture form).
14. If exotic mites are suspected, place all specimens in a labelled plastic or glass vial filled with 80% ethyl alcohol (e.g. methylated spirits). Contact your local department of agriculture entomologist to arrange diagnostics and to determine the preferred method of specimen preparation/preservation prior to sending samples.

15. Report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Refer to *Detection of a suspect exotic pest*, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

16. It has been reported that Varroa mites may be carried on people (D Anderson, CSIRO Entomology, pers comm, November 2004). Therefore, if Varroa mites are detected, decontamination of clothes and body (including hair and beards if exposed) should be carried out.

*Figure 7. Shake a brood comb into a container. Image courtesy Daniel Martin, VIC DEPI.*

*Figure 8. Pouring bees into the mite shaker. Image courtesy Daniel Martin, VIC DEPI.*
Figure 9. Screw the two mite shakers together firmly. Image courtesy Daniel Martin, VIC DEPI.

Figure 10. Shake the mite shaker jars vigorously for around 20 seconds. Image courtesy Daniel Martin, VIC DEPI.

Figure 11. A large number of Varroa mites present. The blue arrows indicate some at the bottom of the mite shaker after settling. Image courtesy Daniel Martin, VIC DEPI.
7.1.3.4 Alcohol washing procedure 2

1. This method does not require a soldering gun to join plastic jars together.
2. Get a plastic jar with an intact lid.
3. Follow the first 6 steps as outlined in Procedure 1.
4. Shake the bees in the jar with the alcohol for 20 seconds.
5. Place a piece of cloth over the top of a small bucket or container, with the 3mm wire mesh slightly over the cloth.
6. Pour the contents of the jar through the mesh and over the cloth. The mesh lid will collect the bees but enable any mites to pass through and be collected on the cloth.
7. Inspect the surface of the cloth thoroughly for mites. A magnifying lens can be used if available.
8. Higher recovery rates of Varroa mite can be achieved by refilling the jar containing bees with water and rinsing the bees once or twice. Two rinses will recover more than 95% of Varroa mites present on the bees.
9. This method does not require a soldering gun to join jars together.
10. Follow steps 12 – 16 in Procedure 1.

7.1.4 Drone uncapping

Up to 85% of Varroa mites in a honey bee colony are found within capped brood cells, with a preference for drone brood. Therefore, uncapping drone brood and examining pupae is another method for detection of Varroa mites. It is recommended that all beekeepers conduct this method as it is rapid and can be carried out easily as part of a routine hive inspection. The disadvantage of this method is that the drone brood are killed. The preference of Varroa for drone brood is strongest in the spring and decreases towards the end of the drone rearing season. Therefore this technique is most sensitive when conducted in early spring.

A designed version of this fact sheet is available at www.beeaware.org.au/surveillancemethods. This may be useful for your own use, or to hand out to assisting hobby beekeepers.

7.1.4.1 Equipment required

- Cappings scratcher or wide blade shearing comb mounted on a handle
- Piece of white paper or cardboard

7.1.4.2 Procedure

1. Push the comb of the scratcher through a patch of capped drone brood and pull a large patch of pupae out all at once (see Figure 12 below). Note that this will kill the drone brood.
2. Uncap drone brood on at least three brood frames from randomly selected hives in the apiary. Uncap about 100 drone brood in total.
3. Examine each pupa for reddish-brown mites, which can be clearly seen against the white bodies of the drone pupa (see Figure 13 below). Mites are easier to see on pupae that have pink eyes rather than those that have taken on adult colouration. Pupae that are younger than the pink-eyed stage are often too soft and fall apart when the scratcher is pulled out.

4. The comb can be tapped over a piece of white paper or cardboard. Mites that do not come out with the pupae may fall onto the card.

5. After removing the drone pupae, check the bottom of the drone brood cells for any mites that may not have attached to the removed pupae (see Figure 14 below).

6. To assist with identification, see “Preliminary identification of suspected exotic mites”, page 33 for descriptions and images of exotic and other common mites.

7. Record the presence or absence of mites in the excel workbook (Data capture form).

8. If exotic mites are suspected, place all specimens in a labelled plastic or glass vial and fill with 80% ethyl alcohol (e.g. methylated spirits), or freeze (in vial or zip-lock bag) for 24 hrs to ensure mites are killed. Contact your local department of agriculture entomologist to arrange diagnostics and to determine the preferred method of specimen preparation/preservation prior to sending samples.

9. Report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

10. It has been reported that Varroa mites may be carried on people (D Anderson, CSIRO Entomology, pers comm, November 2004). Therefore, if Varroa mites are detected, decontamination of clothes and body (including hair and beards if exposed) should be carried out.

Figure 12. Cappings scratcher with pupae removed from drone brood cells. Notice the Varroa mite on the white pupa. Source: Kiwimana.co.nz 2011.

Figure 14. Varroa mites present in the bottom of drone brood cells from which pupae have been removed. Source: CSIRO.

7.1.5 Swarm/feral nest capture and catchboxes

Collection of swarms and/or feral nests in and around high risk ports as well as from catchboxes (including remote catchboxes) placed at high risk locations, supplements sentinel hive surveillance activities. Honey bees collected through these methods should be examined for the presence of exotic mites using the alcohol washing procedure (page 23) and Tracheal mite examination (page 44). Information on swarm/feral nest capture and how to set up catchboxes is provided in sections 7.7.1.1 - 7.7.1.3.
7.1.6 Community involvement in surveillance (BeeForce)

The BeeForce community engagement pilot was designed to test the involvement of urban hobbyist and professional beekeepers in a passive surveillance program for Varroa mites in both Melbourne and Geelong in Victoria. The BeeForce pilot demonstrated not only that an active task force could be enlisted and trained, but that after two years of testing in two separate locations, that all participants were still willing to get involved in such an initiative. The pilot program also demonstrated that if this strong motivation is encouraged and nurtured, then the BeeForce model of community surveillance could provide a reliable task force on which government agencies could draw on, not only for early surveillance initiatives as part of the NBSPS, but also for eradication or surveillance in an emergency response for a honey bee emergency plant pest.

As part of the NBSPS, it is required that hobby beekeeper surveillance is increased through additional sugar shaking and/or alcohol washing at specific high risk ports. These methods have been selected as they are easy to conduct, and do not require testing with chemicals under permit.

Information contained below outlines some simple measures that proved effective for the BeeForce trial program in establishing and maintaining active hobby beekeeper involvement. Where possible, these steps should be followed when establishing hobby beekeeper involvement in high risk ports as part of the NBSPS.

- In high risk port locations make contact with a local beekeeping association, or look on the government database for registered beekeepers in the area. Try to find beekeepers that are as close to the port as possible, or at least within 5 km.
- Try to find 5 – 10 beekeepers in the area who are interested and who seem to be reliable in conducting sugar shaking/alcohol washing on a routine basis (i.e. every 2 months).
- Engage with the local association, or local beekeepers, and work with them by explaining the purpose of the overall program and that additional support, such as routine testing is needed. Routine testing (every 8 weeks) is critical in providing early warning for detecting an exotic pest.
- Explain that beekeepers in and around port areas are in the front line for picking up an exotic pest(s) and that they form a critical component of the honey bee industry and that they should be involved.
- Provide training to the local beekeepers or local association and get engagement to ensure that their commitment is maintained. Face-to-face training is preferred, with follow up fact sheets or manuals distributed to participants.
- Try to identify a local industry champion (i.e. President of the local association) who could assist in organising some other local beekeepers to help out on a routine basis.
- Provide instructional material to the group, explaining the NBPS, why and how it is important, and provide resources for the group such as alcohol washing or sugar shaking kits, biosecurity manuals, fact sheets etc. Ensure that volunteers know how to conduct the surveillance method and how to report their findings.
Get in contact with the local association or volunteers as a friendly reminder when it is time for the next surveillance run.

- **Always maintain an open and clear avenue for communication, feedback and any other suggestions.** This open communication avenue is critical in maintaining their involvement and enthusiasm for the Program.

Some of the main BeeForce principles that will ensure continued success with community and hobby beekeeper involvement include the following:

- **Dialogue:** Ensure a clear and on-going two way communication between yourself and the local beekeeping association or beekeeper.

- **No top down:** Face-to-face training is the most effective means to train participants and the only way to evaluate the level of engagement for the duration of the project. An addition training manual for the BeeForce project is useful and was very well received, but it will not replace the face-to-face training. When conducting face-to-face training, download some pest fact sheets, surveillance method fact sheets and provide alcohol washing and sugar shaking kits.

- **Take feedback into consideration:** Trigger frank two-way discussions with participants and address issues of organisation or lack of communication. Try to figure out what system would work best with the local volunteers.

- **Easy communication pathways:** Find better and easier ways to communicate with participants (organise a monthly email chain, appear at beekeeping club meetings etc.). Consider trying to involve the local association and local beekeeping ‘champions’ as intermediaries and for the program in the area. This will help in consultation with beekeepers in the area, as you could talk to a designated representative who could pass the information back to all participants.

- **Share positive feedback:** Motivate all participants by sharing achievements (newsletters, media releases etc.) and reward with routine social events (BBQ’s, presentations etc.). Be engaged.

- **Educate but don’t pontificate:** Attend or organise 1-2 events with the local beekeeping association each year. If not through a local association, try to get the volunteer participants together in one location. Think about inviting charismatic key speakers (and/or industry champions) to talk about the targeted pest and its possible impact. Highlight the importance that participants are playing for the pest’s early detection. They need to know and be convinced that they are making a difference and their input is valued.

- **Acknowledge:** Recognise the effort that these volunteering participants take out of their daily lives to be a part of the Program. Simple gifts such as a DPI honey bee book, PHA Bee Biosecurity Manual, or a smoker or hive tool out of the Program budget goes a long way in recognising the amount of work that volunteer hobby beekeepers provide for the NBPSP.
7.2 Surveillance methods for detection of Tropilaelaps mites

Surveillance methods for Tropilaelaps mites (*Tropilaelaps clareae* and *T. mercedesae*) include the use of Bayvarol or Apistan acaricide strips and sticky mats in sentinel hives and drone uncapping. As only 3 to 4% of adult mites are reported to attach themselves to adult honey bees, sugar shaking and alcohol washing are unlikely to detect these mites.

7.2.1 Acaricide strips and sticky mats

For the use of Bayvarol or Apistan strips and sticky mats, refer to the procedure described on page 17. Descriptions and images of Tropilaelaps mites are included in “Preliminary identification of suspected exotic mites” below.

7.2.2 Drone uncapping

Up to 97% of Tropilaelaps mites in a honey bee colony are found within capped brood cells. Tropilaelaps mites reproduce in both worker and drone cells, but as with Varroa, there is a preference for drone brood. Therefore, uncapping drone brood and examining pupae is one of the best methods for detection. This method is recommended as it is rapid and can be carried out easily as part of a routine hive inspection. The disadvantage of this method is that the drone brood are killed. For the drone uncapping procedure, refer to page 28. Descriptions and images of Tropilaelaps mites are included in “Preliminary identification of suspected exotic mites” below.

7.3 Preliminary identification of suspected exotic mites

Insects and mites present in Australia and found commonly on sticky mats, could potentially be misidentified as exotics. Therefore it is important to confirm identification with a diagnostic laboratory in the relevant state/territory. Descriptions and images of common mites found in Australia and the exotic Varroa and Tropilaelaps species are included in this section to assist with preliminary identification.

7.3.1 Preliminary identification of Varroa mites (*Varroa destructor, V. jacobsoni*)

If mites are found with a body width greater than length, then Varroa mite should be suspected. Adult females are oval, flat, red-brown and around 1.1 mm long and 1.5 mm wide. Mature males are smaller than females and their bodies remain a light tan colour. Males are usually only found on pupae and remain in the cell. Immature stages (normally found on pupae) are light brown to off white in colour.

Varroa mites could be confused with the Braula fly, Tropilaelaps mites or Pollen mites (see Figure 25).


![Image of Varroa mites](image_url)

*Figure 15. Adult female Varroa mites. Sources: Ken Walker Museum Victoria, PADIL (top image); Scott Bauer, USDA Agricultural Research Service, www.Bugwood.org (bottom image).*

### 7.3.2 Preliminary identification of Tropilaelaps mites (*Tropilaelaps clareae, T. mercedesae*)

Female Tropilaelaps mites are light reddish-brown mites with an oval shaped body, and about 0.96 mm long by 0.53 mm wide. Males are almost as large as females, however they are less sclerotized. In contrast to Varroa, Tropilaelaps mites are fast running and hold their first pair of legs upright, resembling antennae.
Tropilaelaps mites could be confused with the Braula fly, Varroa mites or Pollen mites (see Figure 25).

**Figure 16. Adult Tropilaelaps mite. Source: Food and Environment Research Agency (Fera), Crown Copyright.**

**Figure 17. Varroa mite (left) and Tropilaelaps mite (right). Source: Food and Environment Research Agency (Fera), Crown Copyright.**


7.3.3 Insects and mites that could be misidentified as exotic mites

Insects and mites that are present in Australia and found commonly on sticky mats could be misidentified as exotic mites. These include Braula fly (Braula coeca) and harmless mites associated with honey bees (e.g. Pollen mite). Descriptions and images are included in this section to assist with preliminary identification.

7.3.3.1 Braula fly (Braula coeca)

Braula fly is currently present only in Tasmania. It is red-brown, 0.9 mm wide by 1.5 mm long, covered in spine like hairs with six long legs. The Braula fly is not considered a serious threat to commercial bee keeping as it does not damage or parasitise any stage of the honey bee life cycle. However, its presence may reduce the egg laying capacity of queen bees. If Braula fly is found during honey bee surveillance (e.g. on a sticky mat), it could be confused with an exotic mite such as Varroa or Tropilaelaps. However, it is a notifiable pest for mainland Australia and should be reported immediately.

Figure 18. Braula fly (Braula coeca). Source: Simon Hinkley & Ken Walker Museum Victoria, PADIL.
7.3.3.2 Mites associated with honey bees present in Australia

Mites present in Australia that are associated with honey bees could be confused with exotic mites. These include the Pollen mite (e.g. *Melittiphis alvearius*), Flower mites (e.g. *Afrocypholaelaps africana* and *Hattena* spp.) and Oribatid mites.

Pollen mites such as *Melittiphis alvearius* are the most likely to be confused with external parasitic mites, as they are a specialised bee associate which superficially looks like Varroa mite. Because of this close association they are sometimes found on sticky boards. They feed on stored pollen in hives and are not harmful to honey bees. Due to their small size, a laboratory diagnosis is required to confirm the presence of this mite. The species is almost circular, reddish brown in colour, and smaller than Varroa, measuring about 0.75 mm long and 0.75 mm wide. Images below show the appearance of *Melittiphis alvearius* by electron microscopy (Figure 19), distinguishing diagnostic characteristics of *M. alvearius* (Figure 20 and Figure 21) and comparisons with Varroa mite (Figure 22 and Figure 25), Braula fly and Tropilaelaps mite (Figure 25).

*Figure 19. Pollen mite (Melittiphis alvearius) full body image take on a Nikon Eclipse 80i at x100. The body length is 750µm. Source: Owen Seeman, Queensland Museum.*
Figure 20. Pollen mite distinguishing characteristics of the sterno-genital shield and jugular shield taken at 400x. Source: Owen Seeman, Queensland Museum.

Figure 21. Pollen mite jugular shield taken at 400x. Source: Owen Seeman, Queensland Museum.
Figure 22. Adult female Varroa mite venter (left) in comparison to Pollen mite (Melittiphis alvearius) venter (right). Adapted from Walter et al., (2002). Please Note: images are not to same scale. See Figure 25 for more accurate sizes.

Figure 23. Varroa destructor image taken with a Leica DM2500 at 5x magnification. Source: Jurgen Otto, Department of Agriculture.
Figure 24. Varroa jacobsoni image taken with a Leica DM2500 at 5x magnification. Source: Jurgen Otto, Department of Agriculture.

Figure 25. Braula fly (top), Varroa mite (right), Tropilaelaps mite (bottom) and Pollen mite (left). Source: Food and Environment Research Agency (Fera), Crown Copyright.
Afrocypholaelaps africana is a flower mite common on honeybees in south-east Queensland and northern New South Wales (Seeman & Walter 1995). They feed on pollen and nectar in flowers and use flower visitors such as honey bees to move from one inflorescence to the next (Walter et al., 2002). However they are rarely found in hives.

Figure 26. Adult female Flower mite (Afrocypholaelaps africana) venter. Adapted from Walter et al., (2002).

Figure 27. Dorsal view of a female Afrocypholaelaps taken at x400. Source: Ron Ochoa, USDA.
Afrocypholaelaps has a distinct spermatheca – two tubes entering one large spermatheca. The related genera *Neocypholaelaps* and *Hattena* do not have this, or rather it is not visible. When inspecting sticky mats, you should expect to see *Neocypholaelaps* too, but rarely *Hattena*, which moves between flowers on birds and will only use bees when desperate (Pers. Comm Owen Seeman, May 2014).

*Figure 28. Afrocypholaelaps distinct spermatheca taken at x1000. Source: Ron Ochoa, USDA.*
Oribatid mites feed on a wide variety of material including living and dead plant material, fungal material, lichens and carrion, however, they have been recovered from sticky mats in hives. They are tiny, measuring up to a millimetre long only. They all have a hard exoskeleton but their colour can vary (Figure 29).

Figure 29. Oribatid mites. Source: Ray Norton (top image), www.fcps.edu/islandcreekes/ecology/soil_mite.htm
7.4 Surveillance methods for Tracheal mite detection

Bees from sentinel hives or collected swarms can be examined for the presence of Tracheal mite (*Acarapis woodi*). Due to their very small size (~120 – 180 microns) and presence in honey bee tracheae, Tracheal mite can only be detected with bee dissection and examination under a microscope (see Figure 30).

Two methods can be used for detecting Tracheal mite: individual dissection and examination of tracheae, or examination of stained thoracic discs (see below). The method used will depend on the technical skills, equipment and preference of each laboratory, as well as the number of bees to be examined. Both methods have been adapted from the DPIPWE Entomology Methods Manual (2013).

For testing of sentinel hives for the presence of Tracheal mite, samples are collected from hives at each 8 week visit. Ideally, each sample will consist of approximately 50 bees from a randomly selected hive at each of the port areas. It has been demonstrated that a 1-2% rate of infection can be detected by sampling 50 bees (OIE 2008) and therefore, this should be viewed as the minimum for sampling for Tracheal mite.

Samples of bees should be collected from under the lid of the hive, from outside the cluster or from the hive entrance. Generally, only newly hatched bees under 10 days old are susceptible to Tracheal mite infestation.

Bee samples should be frozen or freshly preserved in 70% ethanol (or similar e.g. methylated spirits). Dead, dried out or decomposing bee samples are unacceptable for dissection and examination for Tracheal mite.

Bee samples are usually sent to Bugs for Bugs for analysis with an accompanying email to Dr Alberto Guanilo (Bugs for Bugs) (alberto@bugsforbugs.com.au) and PHA, including the following information from the Data capture form (workbook):

- Site_num (sentinel hive code)
- Place_date (date of placement of acaricide strips in hive)
- Date_sent (date sample sent to Bugs for Bugs)

The postal address for samples is:

Dr Alberto Guanilo  
Bugs for Bugs  
1 Bowen St  
Mundubbera 4626  
Australia
Figure 30. Tracheal mites (Acarapis woodi) live inside bee airways and are microscopic in size. Source: Simon Hinkley and Ken Walker, Museum Victoria, PADIL.


For more information about another method which only requires a dissecting microscope, see http://www.ars.usda.gov/SP2UserFiles/person/31186/Sammataro-VP(32-4)-November%204.pdf

7.4.1 Individual dissection and examination of tracheae

The following was adapted from DPIPWE (2013) in which methods were incorporated from Shimanuki and Knox (2000), Sammataro (2006) and PHA (2012).
7.4.1.1 Equipment and reagents required

- Dissecting/stereo microscope with sub-stage lighting
- Compound microscope
- Scalpel and disposable scalpel blades (preferably no. 23)
- Fine forceps (preferably curved)
- Microscope slides and cover slips
- Miscellaneous dissection and manipulative instruments
- Fine point permanent markers
- 5-10% KOH solution
- 70% Ethanol
- Pharmacy grade glycerine
- Distilled water
- 1% aqueous methylene blue (prepared by dissolving methylene blue first, then adding sodium chloride to make a 0.85% sodium chloride solution)

7.4.1.2 Procedure

1. Remove the head and forelegs from the thoracic collar by pulling off with a pair of forceps.
2. Remove the abdomen from the thorax likewise.
3. Leave thorax to stand overnight in a 5-10% KOH solution to macerate muscle tissue. May be gently heated the following day.
4. To complete the process, remove tissue, rinse thoroughly in water and place into 70% Ethanol.
5. Working in 70% Ethanol, remove the collar to expose the tracheae. The main thoracic tracheae are dissected out. These consist of the prothoracic spiracle, prothoracic wing and leg tracheae, main posterior thoracic branch, and remainder of head and airs sac branch.
6. Stain in 1% aqueous methylene blue (optional).
7. Rinse in 70% Ethanol, place in glycerine on microscope slide and apply glass cover slip.
8. Examine for the presence of mites under high magnification with dissecting microscope or low magnification with compound microscope.
9. Record the presence or absence of Tracheal mite in the excel workbook (Data capture form).
11. If Tracheal mite is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.
7.4.2 Preparing and examining stained thoracic discs

The following was adapted from DPIPWE (2013) in which methods were incorporated from Shimanuki and Knox (2000), Peng and Nasr (1985) and PHA (2012).

7.4.2.1 Equipment and reagents required

- Dissecting/stereo microscope with sub-stage lighting
- Compound microscope
- Scalpel and disposable scalpel blades (preferably no. 23)
- Fine forceps (preferably curved)
- Microscope slides and cover slips
- Miscellaneous dissection and manipulative instruments
- Petri dishes, including one wax filled petri dish
- Filtration device for moving discs between solutions. Peng and Nasr (1985) recommend a Tissue-Tek perforated processing capsule. Our lab constructed custom filters (Figure 32) using 85 mm x 50mm (h x d) glass jars with plastic lids with 2 x 18mm holes. Synthetic fabric (60 μm monomesh) was fitted under the lid and a hole was cut in the fabric through 1 of the lid holes (pressure release hole) to allow solution flow through the fabric in the other hole (filter hole)
- Oven set at 38°C
- 5% KOH solution
- 70% Ethanol
- Pharmacy grade glycerine
- Hoyers medium
- Distilled water
- 1% aqueous methylene blue (prepared by dissolving methylene blue first, then adding sodium chloride to make a 0.85% sodium chloride solution)

7.4.2.2 Procedure

1. Dissect thoracic discs: Under a dissecting microscope, conduct dissections in a wax filled petri dish:
   - Impale the bee ventral side up with the tips of curved forceps through the mid-coxae (Figure 31A-B).
   - Remove the first pair of legs and head with a scalpel (Figure 31C).
   - Using a sharp scalpel, slice a thin (approximately 1.5 mm), transverse section from the anterior face of the thorax to obtain a disc. Use the forceps to hold the bee and position to guide the scalpel (Figure 31D).
   - Examine the prothoracic spiracles and surrounding area on each disc for signs of mites.
   - Place each disc in 70% Ethanol until the 50+ discs from the sample have been prepared.
2. Clear discs:
• Place the 50+ discs into the custom filter (Figure 32) containing enough 5% KOH to cover the prepared thoracic discs, label with the site/hive number.
• Cover with lid and position the synthetic fabric (60 μm monomesh) so that the hole in the fabric matches one of the holes in the lid (ie pressure release hole shown Figure 32).
• Incubate at 38°C for 24 hours.
• Check that the KOH has dissolved the muscle and fat tissue, leaving the tracheae exposed.

3. Rinse in water: carefully pour the KOH out of the filter hole on the custom filter (Figure 32) (NOTE: do not pour through the pressure release hole at any stage of the process) then replace with tap water, soak for approximately 5 minutes).

4. Stain: carefully pour the tap water out of the filter hole on the custom filter (Figure 32) repeat the rinse if necessary. Replace the tap water with 1% aqueous methylene blue, soak for approximately 5 minutes.

5. Rinse in 70% ethanol: carefully pour the 1% aqueous methylene blue out of the filter hole on the custom filter (Figure 32) then replace with 70% ethanol, soak for approximately 5 minutes, repeat if necessary.

6. Examine discs containing the tracheae in a petri dish containing 70% ethanol under dissecting/stereo microscope with sub-stage lighting, when examining position so that the collar is facing the bottom of the petri dish.
• Stained mites should be readily visible. Remove discoloured or tracheae suspected of containing mites and mount on a microscope slide with a cover slip. Use glycerine for temporary slides or Hoyers for semi-permanent slides.
• Examine prepared slides with a compound microscope for suspect tracheal mites.
• (Optional) If <10 discoloured or suspect tracheae are observed, randomly select 5-10 from each batch of 50 for slide preparation and examination as detailed above.

7. Record the presence or absence of Tracheal mite in the excel workbook (Data capture form).

8. If Tracheal mite is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.
Figure 31. Dissection of *Apis mellifera* for Tracheal mite detection. Source: DPIPWE (2013).

Figure 32. Custom filter. Source: DPIPWE (2013).
7.5 Surveillance methods for Small hive beetle detection

It can be very difficult to detect low numbers of SHB \textit{(Aethina tumida)} in hives and therefore trapping is the most useful tool for early detection. Traps currently available include the Apithor harbourage that contains an insecticide (Fipronil) to kill trapped beetles. Non-chemical traps usually contain oil to drown trapped SHB, although lime or diatomaceous earth can also be used. From 2013, surveillance for SHB was formally integrated into the NBPSP and began in Tasmania and Northern Territory where it is currently not present, as well as through Western Australia where SHB has a restricted distribution (north of Kununurra).

7.5.1 Apithor harbourages

The Apithor harbourage is comprised of two black, rigid moulded plastic shells that hold a Fipronil treated 4 mm corrugated cardboard insert (see Figure 34). This insert is located 10 mm back from the 3 mm wide entrance slots. Size differences between the beetles and the honey bees and the precise dimensions of the harbourage prevent the honey bees from contacting the cardboard insert but allow easy access for SHB (see Figure 35).

The plastic shells are ultra-sonically welded to produce a tamperproof device so that the harbourage can be safely handled without fear of contacting the insecticide. For information on where to purchase and the cost of Apithor harbourages, see \textit{Purchase of chemicals and supplies used in the NBPSP}, page 95.

Apithor is now registered for use in all states and territories of Australia (as of 9/12/2013). For further information, refer to the APVMA registration information,

**Figure 34. Components of an Apithor harbourage prior to assembly. Source: Levot (2008).**

**Figure 35. Dead Small hive beetles inside a deconstructed Apithor harbourage. Source: Levot (2008).**
7.5.1.1 Procedure

1. With a hive tool, paint scraper or similar implement remove wax and debris from a sufficient area of the bottom board to accommodate the Apithor harbourage.
2. Tie some thin gauze wire to the corner of the Apithor harbourage and leave the wire hanging out the front of the hive entrance. This is so the harbourage can be removed from the hive without disturbing the brood box.
3. Place the Apithor harbourage flat surface down, on the bottom board with the slot ends aligned away from the hive entrance.
4. The Apithor harbourage must sit flat on the bottom board so that the SHB cannot shelter underneath.
5. DO NOT use the Apithor harbourage in hives with perforated bottom boards or that are subject to water inundation.
6. Leave the Apithor harbourage on the hive bottom board for 8 weeks. If placing the Apithor harbourage into a sentinel hive, leave on the bottom board for 8 weeks between the testing period of miticide strips for Varroa mites and Tropilaelaps mites.
7. After the 8 week period, remove the Apithor harbourage and place into individual sealable plastic bags (labelled with the sentinel hive code if used) and bring back to the laboratory for diagnostics.
8. If the hive tested is not a sentinel hive and does not have a specific code, record the location that the hive was tested as according to the locations tab in the data capture form.
9. In the sealable plastic bag, shake the Apithor harbourages vigorously and tap on a bench with the slot ends facing down to ensure that any SHB in the trap are dislodged.
10. If any SHB (see Figure 38) or other insects are dislodged from the Apithor harbourage, express post the sealed plastic bag to your local department of agriculture entomologist for diagnostics. For further information on packaging and sending samples, refer to page 92.
11. If SHB (*Aethina tumida*) is positively identified, report this to your jurisdictions Chief Plant Health Manager immediately.
12. Record the presence/absence of SHB in the excel workbook (Data capture form).

7.5.2 Non-chemical traps

Non-chemical traps can be home-made or are commercially available. Most home-made traps are reservoir type devices that provide SHB refuge from bee attack by preventing bee entry. A range of different container types can be used including jars, takeaway food containers, Petri dishes, fishing tackle boxes, etc. Using or making the container opaque encourages SHB entry as they prefer dark places to hide. The trapping devices can be attached or put anywhere within the hive but more success has been obtained by attaching or placing them on the bottom board. Although this type of trap is more effective if it covers the entire bottom board, this is likely to require modifications to the
hives. It is often easier to use a container that fits into or onto the hive so that major hive modifications are not necessary.

SHB entry holes, drilled or melted, should be 4.5 mm in diameter to allow entry by beetles but not bees. To date the most effective products used in the reservoirs have been vegetable oils, agricultural lime or diatomaceous earth. Regularly checking, killing and removing SHB present in the harbourage can help to reduce SHB numbers in the hive. These devices are better suited for beekeepers with stationary hives rather than commercial migratory operations due to spillage, for example, of oil into the hive.

7.6 Identification of Small hive beetle

Larvae are white, about 10-13 mm long and 1.6 mm wide, with 3 pairs of prolegs near the head (Figure 36). Their most distinctive feature is two rows of spines along the centre of the back, with the last two extending beyond the rear end of the larva (Figure 37). Larvae should not be confused with Wax moth (*Galleria mellonella*, *Achroia grisella*) or Dried fruit beetle (*Carpophilus* spp.) larvae which have both been recovered from honey bee colonies. They can however be distinguished by a few features. Wax moth larvae do not have spines, however they have setae (hairs) on segments and a number of prolegs in addition to thoracic legs. In addition Wax moth larvae spin webs or cocoons on frames of hives, in contrast to SHB larvae which cause the honey to ferment and the hive to become ‘slimed out’. Dried fruit beetle larvae are smaller than SHB larvae, measuring less than 8 mm long. They have more dorsal spines per segment and the posterior spines are relatively larger than SHB. They are generally found in debris on the bottom board or pollen stores of dead hives.

Adult SHB are flattened and oval in shape, brown-black in colour, measure about 5 mm long and 3 mm wide, which is about a third the size of a worker honey bee. Distinguishing features include clubbed antennae (Figure 38) and wing cases (elytra) that are shortened so that the apical few segments of the abdomen are visible (Figure 39). Adult SHB could be confused with other adult beetles such as Dried fruit beetle (*Carpophilus* spp.) and Dermestid beetles (e.g. *Dermestes* spp.). Dried fruit beetles are dark brown in colour and are smaller than SHB, measuring about 3.5 mm long and 1.5 mm wide, with an oval, flattened body. They are generally found in debris on the bottom board or pollen stores of dead hives. They move quickly from light but not as fast as SHB. Dermestid beetles are generally found in debris of dead hives and are dark brown-black with a flattened body ranging in size depending on species. For further information about SHB and images of similar looking larvae and adults, see the PIRSA fact sheet located at

Figure 36. Small hive beetle (Aethina tumida) larva. Source: Simon Hinkley and Ken Walker, Museum Victoria, PADIL.

Figure 37. Small hive beetle (Aethina tumida) larva. Source: Simon Hinkley and Ken Walker, Museum Victoria, PADIL.
Figure 38. Small hive beetle (Aethina tumida) adult. Source: James D Ellis, University of Florida, Bugwood.org

Figure 39. Small hive beetle (Aethina tumida) adult. Source: Simon Hinkley and Ken Walker, Museum Victoria, PADIL
7.7 Surveillance methods for detection of pest bees

This section includes available methods for the detection of pest bees, including Asian honey bee (*Apis cerana*), Giant honey bee (*A. dorsata*), Red dwarf honey bee (*A. florea*), and exotic strains of the European honey bee (*A. mellifera*), including Africanized honey bees (*A. m. scutellata*) and Cape honey bees (*A. m. capensis*).

When attempting to locate and destroy swarms or nests of *Apis* spp. in port areas, try to follow the generic guidelines listed below:

- Cavity nesting bees such as *Apis mellifera* or *Apis cerana* can commonly be found in a range of cavities, such as tree hollows or cracks and crevices in buildings. It is also possible to find swarms, and sometimes nests, in more exposed locations such as rolls of cables or on machinery.
- *A. mellifera* and *A. cerana* can colonise a range of cavity sizes, but it is most commonly in the range of 10-15 litres in capacity.
- The height at which to observe nests and swarms can vary greatly from 1 m above the ground, to 10-15 m in a tree. However, most commonly, *A. mellifera* and *A. cerana* will colonise areas around 2 m – 6 m above the ground.
- Look in areas that are protected from large amounts of human, animal or vehicle traffic. Also look in areas that have a close source of pollen or nectar.
- For *A. florea* and *A. dorsata* which have exposed single combs, look in areas that are protected, free from human, vehicle and animal traffic, and look around key overhangs of buildings, warehouses or branches on trees or shrubs.

7.7.1 Surveillance methods for detection of exotic strains of *Apis mellifera*

Exotic strains of the European honey bee (*A. mellifera*), including Africanized honey bees (*A. m. scutellata*) and Cape honey bees (*A. m. capensis*) can be detected through the capture of swarms or feral nests from in and around high risk port areas, from catchboxes or remote surveillance catchboxes placed at high risk port locations. These methods will also pick up established or newly arrived European honey bees present in and around the port area. Any honey bees captured from swarms, nests or catchboxes should be tested for exotic external and internal mites (including Braula fly in mainland Australia).

7.7.1.1 Swarm capture

A swarm is a group of bees searching for a new nesting site. Swarms can be found hanging from any object (e.g. tree branch, house gutter, fence) as a dense cluster around a queen that can vary in size from hundreds to thousands of bees. Swarming usually occurs in spring but sometimes occurs at other times of the year when local conditions permit. They may remain in place for a few hours to up to 1 – 2 days, while scout bees are sent out to seek a new nesting site. Swarming bees are much less
defensive than they would be if still protecting combs with brood and stored pollen and honey.

Regular capture of swarm clusters in and around high risk port areas can lead to early detection of exotic bees. In addition, alcohol washing of captured swarms supplements surveillance activities aimed at early detection of exotic mites as mites can be carried on exotic bees or newly arriving European or Asian honey bee swarms. For early detection of exotic honey bees, all swarms in high risk ports should be captured and tested. The preferred method for swarm capture, adapted from QDAFF (2013a), is provided below.

1. If required, engage a professional pest controller with bee experience to assist in the capture and destruction of a bee swarm or nest.
2. If capturing a swarm yourself, always use personal protective equipment (PPE) and if necessary, seek additional assistance (e.g. a second person on site).
3. Move calmly towards the swarm, then slowly and smoothly place either a large zip-lock bag or a garbage bag (depending on the size of the cluster) around the swarm.
4. Enclose as much of the swarm as you can inside the bag, keeping a firm hand on the bag opening, then squeeze the bag shut, so that very few bees can escape. This is best achieved in one steady and relaxed motion.
5. Gently shake the swarm mass until it drops into the bag.
6. Spray the area where the swarm was originally hanging - this will discourage the bees from reforming on this site.
7. Insert the bag containing the swarm into another bag and tightly secure it with a knot or zip lock.
8. If you did not capture the majority of the swarm, the bees may reform in a mass. If this happens, repeat steps 2–6.
9. Put the bag(s) containing the swarm of bees into a freezer; this will kill the bees. If you cannot put the bees into a freezer, make a small hole in the bag(s) and spray the insecticide into the bag. Seal the hole again and the bees should die within minutes.
10. Walk a safe distance away from the site and have your helper check your clothing for any dead or stray bees. Ensure that these are brushed off before you remove your PPE.
11. Examine the bees to determine if exotic or not (see Identification of exotic bees, page 73).
12. Captured bees (including European honey bees) should be examined for the presence of exotic mites using alcohol washing (see page 23) and Tracheal mite dissection/staining (see page 44). Ensure that the bag in which the bees were contained is checked for the presence of mites that may have fallen off the bees upon placing into the freezer or following insecticide spray.
13. Record the bee species identified and the presence/absence of exotic mites in the excel workbook (Data capture form; BSUR tab; select Swarm collection from Activity drop down list).
12. If an exotic bee or mite is suspected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

7.7.1.2 Feral nest capture

A nest is a permanent colony of bees that may be concealed within a cavity such as a tree hollow, roof, wall vent, container or unused/discarded mechanical equipment. Bees can usually be seen moving consistently in and out of the entrance to the nest as they continue to forage for food. Nesting bees are more aggressive and defensive than swarm clusters as they are defending their nests and queen.

The ad hoc capture of feral nests in and around high risk port areas supplements other surveillance activities being undertaken for the early detection of exotic bees and mites. In addition, alcohol washing of captured nests and drone uncapping of recovered comb, supplements surveillance activities aimed at early detection of exotic mites. The preferred method for capturing a nest, adapted from QDAFF (2013a), is provided below.

1. If required, engage a professional pest controller with bee experience to assist in the capture and destruction of a bee swarm or nest.
2. Always use PPE when capturing a nest and if necessary, seek additional assistance (e.g. a second person on site).
3. Examine the nest entrance and check for evidence of other exits that the bees are using. If secondary exits or cracks are visible, plug these with wet paper towel (or something similar) to stop the bees using these to escape.
4. If the main nest entrance is large, bees could escape out of it when you are spraying. To avoid this, plug the main entrance but leave a small gap so you can aim the spray into the nesting cavity.
5. Spray at least one full can of flying insect killer into the nesting cavity. Try to spray directly onto the comb and nest. After you have sprayed the required number of cans, plug the entire main entrance hole with wet paper towel (or something similar) and leave it for a few minutes. The fumes from the spray will circulate within the cavity and kill the remaining bees. (The amount of spray required to kill a nest depends on the nest size and the location of the cavity)
6. Spray any foraging bees that are trying to return to the nest - they may gather around the plugged entrance.
7. Listen to the noise inside the sprayed nest. When it changes from a high-pitched hum to a low-pitched hum or very little noise, you can presume that the nest is dead.
8. Keep the nest sealed for as long as possible after spraying.
9. If possible, extract the comb and dead bees from the nest and place into a bag, securing it with a knot or zip lock. Store in a freezer or 70% ethanol.

10. If you cannot extract the comb, check the nest the following day to ensure that there are no live bees in or around the nest and to confirm that it is dead. If possible, permanently seal the entrance.

11. If the nest is still active and bees are coming and going from the site, repeat steps 2–8 until the nest is completely dead.

12. After you have finished spraying or checking the nest, walk a safe distance away from the site and have your helper check your clothing for any dead or stray bees. Ensure these are brushed off before you remove your PPE.

13. If bees were recovered from the nest, examine to determine if exotic or not (see Identification of exotic bees, page 73).

14. Captured bees (including European honey bees) should also be examined for the presence of exotic mites using alcohol washing (see page 23) and tracheal dissection/staining (see page 44). Ensure that the bag in which the bees and comb were collected in is checked for the presence of mites that may have fallen off the bees upon placing into the freezer or following insecticide spray. Perform drone uncapping on recovered comb to check for the presence of exotic mites (see page 28).

15. Record the bee species identified and the presence/absence of exotic mites in the excel workbook (Data capture form; BSUR tab; select Swarm collection from Activity drop down list).

16. If an exotic bee or mite is suspected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

### 7.7.1.3 Catchboxes

Catchboxes positioned in high risk port areas can provide a means of early detection of exotic species of *A. mellifera* including Africanized honey bees (*A. m. scutellata*) and Cape honey bees (*A. m. capensis*). Newly arriving swarms of European honey bee (i.e. inadvertently imported on cargo/vessels) as well as the local *A. mellifera* population may also be picked up using catchboxes and can subsequently be sampled for exotic mites on a regular basis. Catchboxes were shown to be ineffective for picking up Asian honey bee during the Transition to Management program in Cairns (QDAFF, 2013b). Catchboxes will not pick up Giant honey bee (*A. dorsata*) or Red dwarf honey bee (*A. florea*) as they are not cavity-nesting honey bees.

The procedure for establishing and monitoring catchboxes is provided below.
1. The positioning of catchboxes is very important as it needs to represent a likely home for a swarm of bees – the catchbox should be about 40 litres in volume, be secure (i.e. no holes), have a defined entrance and be placed away from direct human, animal or machinery traffic. It should be placed in a protected (away from strong winds), partly shady area away from direct and intense sun.

2. It is recommended to place at least 10 catchboxes at each high risk port location.

3. If honey bees are present in the catchbox, wait till night time and seal the entrance.

4. Once the entrance has been sealed, there are two options to kill the bees present and retrieve the bees for diagnostics.
   a. Place the sealed catchbox in a bag and place in a chest freezer (-18°C) for at least 24 hours to kill the bees.
   b. If a quick turn-around is required, seal the catchbox and then bag it. Get a cylinder of CO₂ and release CO₂ into the catchbox. Seal the entrance again and wait for the humming to subside. Once the bees have quietened down, open the catchbox and remove the bees and/or comb. If the bees are still active, follow the chest freezer method.

5. Please note: Try not to use insecticide in the catchbox, as this may deter bees from entering the catchbox once set up again.

6. Once the swarm has been killed, collect the dead honey bees and any comb (if present) into a plastic bag and tightly secure it with a knot or zip lock. Collect any debris from the catchbox as exotic mites may have fallen off the dead bees onto the catchbox floor.

7. Store bees and comb in a freezer or 70% ethanol. It is not recommended to use insecticide to kill honey bees in the catchbox as this will deter honey bees from entering the catchbox in the future.

8. Examine the bees to determine if exotic or not (see Identification of exotic bees, page 73).

9. Captured bees (including European honey bees) should also be examined for the presence of exotic mites using alcohol washing (see page 23) and tracheal dissection/staining (see page 44). Perform drone uncapping on recovered comb to check for the presence of exotic mites (see page 28).

10. Record the bee species identified and the presence/absence of exotic mites in the excel workbook (Data capture form; BSUR tab; select Catchbox from Activity drop down list).

11. If an exotic bee or mite is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.
7.7.1.4 Remote surveillance catchboxes

A remote surveillance catchbox is an empty hive with a mobile phone camera and sensors that can detect when honey bees are present in the hive. The phone captures an image on a frequent interval and performs image analysis to determine the presence of a swarm. The phone uploads an image on a daily basis or if activity is detected by image analysis. The phone will not analyse an image when the catchbox lid is open. Power to the phone is provided by a solar panel and batteries in the catchbox lid. An electronic door on the catchbox entry can be triggered remotely to close and open the door.

If honey bees are detected, a signal is sent to the NBPSP database where the administrator (PHA) is notified and can confirm the presence of honey bees by inspecting the images sent from the camera. If honey bees are present, the administrator can remotely close the entrance door to the catchbox at night. The jurisdictional coordinator is then notified and is required to go to the remote surveillance hive to kill the bees and undertake the appropriate testing and notification.

The advantage of remote surveillance catchboxes is that they reduce the cost and time required for inspectors to regularly travel to check catchbox sites. In addition, since they can be monitored from a distance, they enable the use of catchboxes at high risk remote locations where it would not normally be feasible to check catchboxes regularly, or in port areas where continued access is hard to achieve. Another advantage is that the administration can take place from a central location (PHA), and therefore a large number of catchboxes can be overseen by a single administrator from one location.

The procedure for establishing and monitoring remote surveillance catchboxes is provided below.

1. The positioning of remote surveillance catchboxes is very important as it needs to represent a likely home for a swarm of bees – the remote surveillance catchbox should be placed away from direct human, animal or machinery traffic. It should be placed in a partially sunny area and away from a windy position. The catchbox should be installed level and strapping or brackets may be appropriate to secure the catchbox in place.
2. If bees are confirmed to be present in the remote surveillance catchbox, the administrator monitoring the catchboxes will close the entrance door to the catchbox at night time and notify the relevant jurisdictional coordinator.
3. Once the entrance has been sealed, the electronics in the catchbox need to be recovered as follows:
   a. Open the catchbox lid. The inner surface of the catchbox lid contains electronic components. The rest of the catchbox is covered by an inner lid (or false ceiling). Unplug the electronic connectors in the catchbox lid that originate from the inner lid. Slide the catchbox lid off at the hinges, to completely remove the catchbox lid from the inner lid and the rest of the catchbox. Set aside.
   b. Unscrew the inspection hole door at the bottom of the catchbox. Release the phone from its strapping, and disconnect the electronic connectors
that connect to the phone. Set the phone aside and replace the inspection
door.

4. Once the electronics have been removed, there are two options to kill the bees
present and retrieve the bees for diagnostics.
   a. Place the sealed catchbox in a bag and place in a chest freezer (-18°C) for
   at least 24 hours to kill the bees.
   b. If a quick turn-around is required, seal the catchbox and then bag it. Get a
cylinder of CO₂ and release CO₂ into the catchbox. Seal the entrance again
   and wait for the humming to subside. Once the bees have quietened
down, open the catchbox and remove the bees and/or comb. If the bees
   are still active, follow the chest freezer method.

5. Dead bees should then be retrieved at a suitable time (i.e within 48 hours).

6. Collect dead bees into a plastic bag and tightly secure it with a knot or zip lock.
Collect any debris from the catchbox as exotic mites may have fallen off the dead
bees onto the catchbox floor.

7. Store bees and comb in a freezer or 70% ethanol. It is not recommended to use
insecticide to kill honey bees in the catchbox as this will deter honey bees from
entering the catchbox in the future.

8. Examine the bees to determine if exotic or not (see Identification of exotic bees,
page 73).

9. Captured bees (including European honey bees) should also be examined for the
presence of exotic mites using alcohol washing (see page 23) and tracheal
dissection/staining (see page 44). Ensure that the bag in which the bees and comb
were collected in is checked for the presence of mites that may have fallen off the
bees upon placing into the freezer.

10. Record the bee species identified and the presence/absence of exotic mites in the
excel workbook (Data capture form; BSUR; select Remote Surveillance from Activity
drop down list).

11. If an exotic bee or mite is detected, report the finding immediately to the relevant
state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084
881) or by directly reporting to the state/territory Chief Plant Health Manager.
Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of
samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest,
page 86 for further details on the procedure to follow if a suspect exotic pest is
detected and how to send samples.

12. Once the bees have been removed from the remote surveillance catchbox, it must
be placed back in its original position and be made 'operational' again. In order for
this to happen you must secure the phone in its strapping in the bottom corner of
the catchbox, and reconnect the electronic connectors to the phone. Replace the
catchbox lid on its hinges and reconnect the electronic connections to the catchbox
lid. Close the catchbox lid. Once complete, contact PHA as the administrator to
determine the image feed from the catchbox has begun again.
7.7.1.5 Floral sweep netting

Structured surveillance methods for pest *Apis* species have historically been difficult to develop and/or implement. Surveillance techniques proposed during the Asian honey bee Transition to Management Program (AHB T2M) in Cairns were shown to be either not effective at picking up low levels of infestation (i.e. log traps, catchboxes), labour intensive (i.e. feeding/sugar stations) or the only options available in regionalised areas of Northern Australia (i.e. rainbow bee-eater pellets). From the work conducted by Biosecurity Queensland and the Northern Australian Quarantine Strategy (NAQS), floral sweep netting represented the most efficient and effective sampling method to confirm the presence of Asian honey bee in the Cairns port area.

To document the methods used, NAQS created a floral sweep netting manual. This manual provided an overview of how floral sweep netting was used to detect the presence of Asian honey bee, as well as the methods and resourcing required to achieve a reasonable level of confidence in the presence or absence of Asian honey bee in a port environment (Rice, 2013).

Considering the research conducted by NAQS, and the reality that another incursion of pest *Apis* spp. poses a significant risk to Australia’s honey bee and pollination-reliant industries, floral sweep netting has been proposed as the main surveillance method to provide a means of early detection.
Unfortunately, there are no international standards or guidelines for floral sweep netting as a surveillance method for high priority exotic pest bees. For this reason, PHA as coordinators of the NBPSP, have created the following guideline for delivery at high risk ports around Australia as part of the NBPSP. The methods outlined below build upon the research conducted by NAQS (Rice, 2013), but also summarise the best available research from relevant peer reviewed articles and from discussions with experts in the field.

The guideline provides instruction regarding the resourcing requirements of floral sweep netting at a port, including when to conduct floral sweep netting, how often and for how long. This has been compiled to ensure the NBPSP has a surveillance method for early detection of high priority pest *Apis sp.* in Australia, including the Red dwarf honey bee (*Apis florea*), the Giant honey bee (*Apis dorsata*) and exotic and established strains of Asian honey bee (*Apis cerana*). This method is not meant to provide a means for detection of the Africanised honey bee (*Apis mellifera scutellata*) or the Cape honey bee (*Apis mellifera capensis*).

**Identifying *Apis* species**

As with any insect surveillance, recognising target from non-target species is essential. Differentiating *Apis cerana*, *A. florea* and *A. dorsata* from local insects, including the established European honey bees (*Apis mellifera*) is not usually a difficult task and most people can become adept at identification with training and experience. It is recommended, however, that surveillance for pest *Apis* spp. is supported by an experienced entomologist beyond just the confirmatory diagnostics.

**How to create a floral map around a port**

Follow the below steps to create a floral map around a port:

- Establish a target area around the port vicinity. This will constrain the workload to manageable levels and allows targeting of the highest risk areas.
- The target zones should be reflective of the likely entry points of vessels into the port, including truck or rail terminals and will aid in detecting a new incursion of pest bees.
- Map an area roughly 600 m from the main port facilities and target zones (i.e. likely points of entry for pest bees). Please see *Foraging ranges and partitioning* section below for more information about why 600 m has been selected as the target range for *Apis* spp.
- A shape file can then be uploaded to a GPS or an i-pad to allow officers on the ground to stay within the zone whilst mapping floral resources (see Figure 41 as an example). If this is not available, use Google maps, or another mapping system to determine the range from the port vicinity. If an electronic means is not available to map the distance from the port, simply print off a detailed map of the port with a 600 m radius drawn in.
Once the map has been created, the next stage involves mapping the location and species composition of the floral resources within the zone. This can be done to a very detailed level, or at a basic level. Some ports may contain a large amount of floral resources, while others may contain very little. Ideally, this would involve a botanist and/or a beekeeper to help establish some baseline knowledge of the plant species, and their suitability to bees (nectar and pollen suitability) in the port area.

This approach is more time consuming at the start of the floral sweep netting process, but the data gained can save a lot of time later and involves mapping floral sources that are currently flowering and also those that are likely to flower at different times of the year.

The presence/absence of European honeybees (Apis mellifera) is a good guide as to whether the flowers will be a good floral source for other Apis spp., as they would generally have very similar resource requirements (Pers. Comm. Ben Oldroyd, February 2014). A natural outcome of this work would be a ‘floral target list’ that would include the majority of the flora that are visited by bees within the zone and would include weeds, trees and cultivated garden plants.

Detailing plant species and their location in the port vicinity will help guide future floral sweep netting in the port area, as well as help guide surveillance if a pest is detected.

When mapping floral resources include key details such as scientific name, common name, time of flowering, attractiveness to bees etc. and their location using key place markers such as local landmarks or street names. If possible, also include the GPS location. See Method section below for more information about how to record these details.
How to conduct floral sweep netting around a port

Follow the below steps to conduct floral sweep netting around a port:

- Once the zone is established and a floral map has been created, the most efficient method for ongoing floral sweep netting surveillance is to limit the hours allocated to the task, and a balance can then be found between resource commitment and confidence in surveillance outcomes (Rice, 2013).

- A single snapshot gathered in one floral sweep netting survey is unlikely to give the necessary confidence level of the absence/presence of *Apis* spp. in the port vicinity. For Asian honey bee in the Cairns region, the NAQS team found that Asian honey bee was sometimes hard to find even in areas where there was a well-established population (Rice, 2013). However, if floral sweep netting was conducted as part of a structured surveillance program, then confidence of Asian honey bee presence/absence could be determined over time.

- For Asian honey bee in the Cairns region, the NAQS team also found that once the floral sources within a target zone had been identified and located, three hours was more than sufficient to cover the majority of flowering areas that Asian honey bee was likely to visit (Rice, 2013).

- In summary, the NAQS team observed that the combination of a structured surveillance program for floral sweep netting and an appropriate time to conduct the method provided an effective method of detecting Asian honey bee in the Cairns port area.

Resource and time requirements

The resource and time requirements include the following:

- Each floral sweep netting surveillance run should be conducted for 3 hours every 2 months, in accordance with the regular testing schedule of the NBPSP.

- Ideally, this 3 hour surveillance run should be conducted between 7am – 12pm as this is believed to be the most effective ‘window’ to detect any possible *Apis* spp. in the vicinity (see Foraging ranges and partitioning section below for more information).

- The floral sweep netting surveillance should attempt to be completed on a clear day, with minimal rain, wind etc.

- This surveillance run should be completed within 600m of the port vicinity and major docking areas of the port.

Equipment required

The list of equipment required to conduct floral sweep netting is outlined below:

- Both a long handled and short handled net. The best nets for surveillance for *Apis* spp. are long nets with narrow ends which can easily be flicked over.

- 80 ml vials with about 20 ml of 70% alcohol.
• GPS or I-pad.
• Appropriate equipment and protective clothing for working outdoors and for possibly handling bees.

Method

Follow the below method to correctly undertake floral sweep netting:

• Once the zone around the port is established and a floral map has been created, travel to the areas known to have flowering plants.
• Look for the presence of any feeding insects such as Apis spp. on the floral resource.
• Depending on the height of the floral source, use the short/long handled net to sweep in a figure of ‘8’, continuously moving the net and keeping the opening slightly facing downwards.
• Attempt to do 10-20 floral sweeps per floral source/plant.
• If an Apis spp. is detected or suspected swing the net over the top of the bee and bring it quickly to the ground.
• Bees generally fly upwards in a net, so hold the apex of the net in the air while the loop and handle are on the ground.
• Keep holding the top of the net with one hand, and put the specimen jar up inside the net with the other hand until the bee is in the specimen jar. Hold the netting tight against the entrance of the jar.
• Tap the netting at the entrance of the vial. The bee should fall into the ethanol and die almost immediately. Remove the jar from the net and put the lid on it. This jar can be continually reused until you have up to 30 bees in ethanol.
• Write out a label with a pencil and paper and place into the jar. The label needs to contain the site, date, collector and host(s) e.g.

Port Botany – Botany Lawn Cemetery
February 26, 2014
Collector – Doug Sommerville, NSW DPI
Host – bottlebrush near entrance to venue

• Store specimens in a cool place (e.g. chilled esky, cardboard box out of sunlight).
• Get the specimens examined by a qualified entomologist.
• Complete this method for 3 hours around the port vicinity. Continue to survey a variety of flowering plants, as some plants will produce nectar/pollen at different times of the morning, and therefore, these plants may be attractive to bees at different times of the morning.
• The job is made easier using a vehicle and two staff, enabling one to drive while the other searches for hosts. However, if an accurate floral map has been created
which outlines flowering species at sections around the port, one person should be sufficient for the task.

- A reference collection that includes local species that could be mistaken for *Apis* spp. is worthwhile considering as it is relatively easy to demonstrate the differences to other staff when training them in the method conducted.

- Examine collected honey bees to determine if exotic or not (see *Identification of exotic bees*, page 73).

- Record the honey bee species identified in the excel workbook (Data capture form; BSUR tab; select floral weep netting from Activity drop down list).

- If an exotic bee is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to *Detection of a suspect exotic pest*, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

**Foraging ranges and partitioning**

This summary is based on an excerpt from Oldroyd and Wongsiri (2006).

The typical foraging ranges between *Apis cerana*, *A. florea* and *A. dorsata* can vary. Since each of these species are being targeted as part of the NBPSP, their foraging range has been taken into consideration when proposing the floral sweep netting methodology. As outlined in more detail in Oldroyd and Wongsiri (2006), Dyer and Seeley (1991) demonstrated that half of the *A. cerana* colonies indicated foraging sites at less than 196 m from the colony, 269 m for *A. florea* and 864 m for *A. dorsata*. In *A. cerana*, 95% of dances indicated food less than 1 km from the colony, 1.5 km for *A. florea* and 4 km for *A. dorsata*. Some dances documented as part of this study suggested foraging places over 15 km for *A. florea* and 21 km for *A. dorsata*, however, these were rare.

In a separate study conducted in Japan, the average foraging range for *A. cerana* was estimated to be 2.1 km (Sasaki *et al.* 1993). Research conducted by Commerford and Koetz (2013) in Australia as part of the AHB T2M program indicated that the majority of Asian honey bee nests foraged within 400 m of the nest, and that the Asian honey bee displayed peak pollen foraging time in the morning, but no peak nectar foraging time.

Foraging ranges were discussed in more detail in Oldroyd and Wongsiri (2006), but the distances listed from the Dyer and Seeley (1991) research here are generally reflective of the research in this area. This suggests that all *Apis* spp. are capable of great foraging ranges if required, but will generally work within a few hundred metres of their nest (Oldroyd and Wongsiri, 2006).

As outlined in Oldroyd and Wongsiri (2006) the foraging times of *Apis* spp. can vary considerably, and this can influence when the most appropriate time is to conduct surveillance. Apart from general environmental conditions, such as whether the day is...
clear or rainy, which would determine when a honey bee would begin foraging, it is has been demonstrated that in order to fly a honey bee needs to achieve a thoracic temperature of at least 27°C (Dyer and Seeley, 1997). This ability is dependent on the ambient temperature, colony temperature and the forager’s ability to achieve this via the production of metabolic heat. Because their nests are multi-combed and somewhat insulated, cavity nesting bees such as *A. cerana* and *A. mellifera* can maintain a higher proportion of foragers above the critical 27°C than the open nesting species, therefore providing the ability to forage earlier in the morning, sometimes as early as dawn (Oldroyd and Wongsiri, 2006).

*A. dorsata* generally arrive second, presumably because they are larger bees that live in large colonies, and are therefore able to generate sufficient metabolic heat to be able to release foragers on cool mornings despite being open nesting. *A. florea* generally arrive later in the morning because of the small size of the bee and the open exposed nest.

Different species of bee may forage over different parts of the plant (Oldroyd and Wongsiri 2006). This is because some bees may not be able to exploit the less profitable nectar secreting flowers on a bush. Alternatively, some bees (such as the larger bees which may be susceptible to overheating when foraging in direct sunlight) prefer to forage in the shadier parts of the floral resource. These observations have been confirmed in the Cairns region by both Biosecurity Queensland and NAQS (Commerford and Koetz, 2013; Rice 2013)

We conclude that 600 m is a sufficient distance around a port environment to conduct floral sweep netting for *A. cerana*, *A. florea* and *A. dorsata* that will give some confidence of detection if they are present. Furthermore, surveillance to be conducted for at least 3 hours in the timeslot of 7am – 12am would provide the most effective window for detection.

### 7.7.2 Surveillance methods for detection of Asian honey bee (*Apis cerana*)

Asian honey bee (*A. cerana* Java Genotype) is currently established in the Cairns region in the state of Queensland. However there are exotic strains not currently present in Australia which pose a risk to honey bees as they are not only pests in their own right, but have the potential to carry exotic mites with them if they enter the country. In addition, the strain present in Queensland is not as aggressive as some exotic strains of *A. cerana*. Early detection of new incursions of Asian honey bee and testing for exotic mites may prevent establishment of these more aggressive strains and the exotic mites that may be carried on them.

Asian honey bees can be detected through the capture of swarms or feral nests from in and around high risk port areas, as well as floral sweep netting or rainbow bee-eater (*Merops ornatus*) surveillance.

A report detailing the efficacy of detection methods for Asian honey bee during the Transition to Management program in Queensland concluded that catchboxes were
ineffective for picking up Asian honey bee (QDAFF, 2013b). The report also noted that rainbow bee-eater surveillance was the most efficacious method of detection where roosts were present. In terms of bees found per person per hour, it was 10 times more effective than floral observations and 36 times more than sugar feeding traps (QDAFF, 2013b). Despite this efficiency, roosts are usually not present in and around port areas. However, they are present throughout towns and cities in Northern Australia, and it may therefore provide a useful method for detection of Asian honey bee.

For methodology on swarm, feral nest capture and floral sweep netting, see sections 7.7.1.1, 7.7.1.2 and 7.7.1.5 respectively.

### 7.7.2.1 Rainbow bee-eater pellets

Rainbow bee-eater pellets provide a tool for determining the presence of Asian honey bee (*A. cerana*). The method was developed following the 1998 incursion of *A. cerana* in Darwin and further developed to be used during the eradication efforts of the 2007 Cairns incursion.

The rainbow bee-eater (*Merops ornatus*) is widespread across much of mainland Australia and occurs on several near-shore islands. It is absent from Tasmania, and is thinly distributed in the most arid regions of central and Western Australia. It breeds throughout most of its range, being present in many northern locations throughout the year. The birds in southern Australia, however, migrate north during the winter months, resulting in larger populations in northern Australia between March and November. The birds roost at night in large flocks, and return to the same trees nightly and annually. Although rainbow bee-eaters eat a variety of insects, their diet consists mainly of bees and wasps. When roosting, they regurgitate non-digestible parts of their prey (such as bee wings) in the form of a pellet. As pellets fall to the ground, they can be collected and the contents examined for the presence of *A. cerana* wings (Bellis and Profke 2003). The rainbow bee-eater will forage over a range of several kilometres and may collect bees from areas inaccessible to humans. These features make rainbow bee-eaters useful for establishing the presence of *A. cerana* in an area.

Disadvantages of the surveillance technique include that it requires the presence of a local population of rainbow bee-eaters. For early detection purposes, this local population should ideally be situated as close as possible (within a few kilometres) to a high risk port area. Furthermore, the identification stage is labour intensive and requires a great deal of technical skill. One of the significant unknowns about bee-eaters is the length of time between ingestion of bees by the bird and the disgorging of the pellet. As populations may move seasonally, it is possible that a migrating bird could contribute into the testing system a pellet representing a bee population hundreds of kilometres away. A bird flying south from, say, Saibai Island in the Torres Strait could be disgorging pellets rich in *A. cerana* in Cairns within a day or two.

The following procedure for conducting rainbow bee-eater surveillance was adapted from QDAFF (2013c).
Locating roosting sites

Locating the rainbow bee-eater roosting sites requires the ability to identify its distinctive call (audio file available at http://birdlife.org.au/bird-profile/rainbow-bee-eater). Once the call has been identified, the observer should move reasonably quickly about the area, preferably on a bicycle. Watch for flocks in the late afternoon, as they will begin grouping together in an area. They will then usually begin flying, in stages, towards their chosen roost. Listen for the bird calls and follow these small groups of birds to the roosting site.

Alternatively, ask local bird watching groups if they are aware of roosting sites, or use media to encourage the community to report any known locations.

If roosting sites are difficult to locate or access, birds can be also be found perched on power lines and fences during the day, and pellets can be collected from beneath these areas.

Figure 42. Rainbow bee-eater (Merops ornatus). Source: JJ Harrison, Wikimedia Commons.

Collection of pellets

1. Once the roosting site has been located, spread a white sheet (double bed size) around the base of the tree and peg it to the ground. If suitable, leave overnight to collect as many pellets as possible. Do not use a tarpaulin as this will enable water to pool, disintegrating the pellets. If the site is not suitable for a sheet, pellets can
be collected directly from the ground of roosting sites or beneath birds perched on fences and power lines, if seen regurgitating pellets during the day.

2. Collect pellets into a labelled vial, recording the time, date, locations and any other relevant details. If there are only a few pellets, collect them all. If there are too many present to collect them all, ensure that the pellets are collected to represent all parts of the sheet. Keep pellets dry and do not refrigerate.

3. The frequency of pellet collection will depend on the situation. To confirm the presence of a new AHB incursion, weekly collection is necessary. To determine if an area is free of pest bees, monthly collection is sufficient.

4. Regularly visit each roost site at dusk to confirm they are still active and look for any variation in the most favoured tree at the site.

5. Laboratory analysis is required to determine whether exotic bees are part of the rainbow bee-eaters diet, in which case wings of the exotic species will be present in the pellets. The wings found, are usually dry and curled up and therefore a laboratory is required to extract the wings from a pellet and identify the species present.

### 7.7.2.2 Extracting wings from a pellet

1. Place pellet into small vial (50 – 100 mL) and pour water over the contents, replacing the screw top to tightly seal the vial.
2. Gently shake vial to loosen material within.
3. Pour contents of vial into tea strainer or fine mesh sieve.
4. Rinse the contents under cold water.
5. Place contents into a petri dish and cover material with 70% ethanol.
6. Remove bee wings from dish and identify if the species present is *A. mellifera* or *A. cerana*, by comparing wing venation patterns as described in section 7.8.
7. If *A. cerana* is positively identified, report this to your jurisdictions Chief Plant Health Manager immediately.
8. Record the presence/absence of *A. cerana* in the excel workbook (Data capture form).
Figure 43. Collecting bee-eater pellets from sheets spread on the ground beneath a roosting tree. Source: Animal Health Australia.

7.7.3 Surveillance methods for detection of Giant honey bee (*Apis dorsata*) and Red dwarf honey bee (*Apis florea*)

The Giant honey bee (*A. dorsata*) and Red dwarf honey bee (*A. florea*) can be detected through the capture of swarms or feral nests from in and around high risk port areas or through floral sweep netting. For methodology on swarm, feral nest capture and floral sweep netting, see sections 7.7.1.1, 7.7.1.2 and 7.7.1.5 respectively. For details on identifying *A. dorsata* and *A. florea*, see Identification of exotic bees below.

7.8 Identification of exotic bees

Bees captured from catchboxes, swarms, feral nests and floral sweep netting undertaken in and around the port areas should be examined to determine if they are exotic and/or are carrying any bee pests.

1. For species identification of samples from a single catchbox or swarm capture/feral nest, examine a minimum of 10 bees in a 50 bee sample. For samples originating from multiple or an unknown number of sources (e.g. if sweep netting), examine at least 50 bees.
2. Spread bee sample over a large petri dish.
3. Use keys provided below to identify species present. Use a dissecting microscope if available.
4. Wing venation patterns provided below can also be used to distinguish European honey bee (*A. mellifera*) from Asian honey bee (*A. cerana*).
5. For more information about how to differentiate between Asian honey bee and the European honey bee, please see QDAFF (2013c) *Asian honey bee manual*:

6. Following species identification, all bees recovered from catchboxes or swarms/feral nests should be alcohol washed (see page 23) to determine the presence/absence of exotic external mites. Bees should then be stored in 80% ethyl alcohol (e.g. methylated spirits) and at least 50 should be examined for the presence/absence of Tracheal mite (see page 44).

7. Record the presence/absence of exotic bees and mites in the excel workbook (Data capture form).

8. If an exotic bee or mite is detected, report the finding immediately to the relevant state/territory agriculture agency through the Exotic Plant Pest Hotline (1800 084 881) or by directly reporting to the state/territory Chief Plant Health Manager.

9. Contact a diagnostic laboratory in the relevant jurisdiction to arrange sending of samples for confirmatory diagnostics. Refer to Detection of a suspect exotic pest, page 86 for further details on the procedure to follow if a suspect exotic pest is detected and how to send samples.

A basic guide for the identification of Apis cerana, A. florea, A. dorsata and A. mellifera

Bees are of the order Hymenoptera (which includes wasps, ants and sawflies) and the suborder Apocrita. There are four main families of bees in Australia that include over 1500 native bee species:

1) Apidae
2) Colletidae
3) Halictidae
4) Megachilidae

Some wasps, flies and native bees can be easily misidentified as Apis spp. due to a similar appearance, especially by inexperienced field staff and by the community. Therefore, it is always critical that formal identification is undertaken by a trained entomologist on any suspect sample found in the field.

A basic identification guide on how to determine if a suspect sample could be an Apis spp. is listed below. Simple characteristics and photos of foragers of the four main Apis spp. are also listed below to provide some very basic guidelines on their identification.

Physical Characteristics

All of the Apis species targeted in floral sweep netting belong to the Apidae family of bees. A series of simple morphological characteristics can be used to determine if a bee belongs to the Apidae family.
1) The antennae must have a bend after the first long segment, like an elbow as demonstrated in Figure 44. *Apis* spp. also have hairy eyes, while only a few native bees have this characteristic.

2) All bees have two pairs of wings – the upper is the forewing and the lower is the hindwing, as demonstrated in Figure 45. The wings are joined together by a series of hooks on the front part of the hind wing and a groove on the back part of the forewing.

3) The forewings of all species of the Apidae family have three cells (called submarginal cells), each with the characteristic shape shown below in Figure 46.
Figure 46. Forewing venation pattern of an Apidae family bee. Source: PADIL.

There are four main species of *Apis* that are being targeted during floral sweep netting. These include the Giant honey bee (*Apis dorsata*), European honey bee (*A. mellifera*), Asian honey bee (*A. cerana*) (Figure 47) and Red dwarf honey bee (*A. florea*).

Figure 47. Lateral view of *Apis dorsata*, *Apis mellifera* and *Apis cerana*. Source: PADIL.
Differentiation characters for the *Apis* spp. which are being targeted during floral sweep netting are listed below in Table 2. An explanation of the tomatical hair characteristic and cubital index information is contained below.

**Table 2. Differentiation characters between the four main *Apis* spp. Source: PADIL**

<table>
<thead>
<tr>
<th>Character</th>
<th><em>A. mellifera</em></th>
<th><em>A. cerana</em></th>
<th><em>A. dorsata</em></th>
<th><em>A. florea</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Forewing length (mm):</td>
<td>8.0-9.7</td>
<td>7.4-9.0</td>
<td>12.5-14.5</td>
<td>6.0-6.9</td>
</tr>
<tr>
<td>Cubital index:</td>
<td>1.65-2.95</td>
<td>3.1-5.1</td>
<td>6.1-9.8</td>
<td>2.8-3.7</td>
</tr>
<tr>
<td>Tomental hair:</td>
<td>T3-5</td>
<td>T-3-6</td>
<td>T3-6</td>
<td>T3-6</td>
</tr>
<tr>
<td>Nest:</td>
<td>Several combs in cavities</td>
<td>Several combs in cavities</td>
<td>Single large comb at bottom of branch or projecting rock</td>
<td>Single small comb that fully surrounds a small branch or twig</td>
</tr>
</tbody>
</table>

**Tomental hair**

Tomentum is defined as hair pressed close to a surface. ‘Tomental hair’ is any hair that appears as distinct white or yellow bands either across the abdominal segments or appears only on either side of the abdominal segments (Figure 48).

![Image of a bee showing tomental hair](image_url)

**Figure 48. Tomental hair bands extending across the entire width of each abdominal segment. Source: Ken Walker, Museum Victoria.**
Cubital Index

In the forewing of a honeybee, the cubital cell is crossed basally by the 1st recurrent vein. The cubital index is the distance to the left of where the 1st recurrent vein crosses the basal vein of the cubital cell to the right margin of the submarginal cell. That measurement is then divided by the distance to the right of where the 1st recurrent cell crosses to where the cubital cell recures back up.

![Cubital Index Diagram](image)

Figure 49. How to determine the cubital index in the forewing of a honeybee. Source: Ken Walker, Museum Victoria.

Dwarf honey bees (*Apis florea* and *A. andreniformis*)

The Red dwarf honey bee (*Apis florea*) is red-brown and has quite distinct red/brown and white and black bands on the abdomen. A foraging worker bee body length is between 7 - 10 mm long, while the forewing length is between 6.0 - 6.9 mm in length. A closely related exotic species, *A. andreniformis* (Black dwarf honey bee) is roughly the same size, but is blacker in colour, as the name suggests. See Oldroyd and Wongsiri (2006) for more descriptions.

For images on diagnostic features, click the following PADIL link http://www.padil.gov.au/pests-and-diseases/Pest/Main/135537
The Giant honey bee (Apis dorsata)

The Giant honey bee (Apis dorsata) is comparatively very large (~17mm long) and their colour is quite similar to the European honey bee, with golden, black and pale bands on the abdomen and with a hairy thorax. Their forewing length can vary from between 12.5 – 14.5 mm. A closely related giant honey bee, the Giant Philippine honey bee (Apis brevilligula) may also be observed. See Oldroyd and Wongsiri (2006) for more descriptions.
For images on diagnostic features, click the following PADIL link http://www.padil.gov.au/pests-and-diseases/Pest/Main/135534

Figure 52. Forager Giant honey bee on sunflower. Source: Sam Malfroy, Plant Health Australia.

Figure 53. Giant honey bee next to three foraging Red dwarf honey bees. Source: http://www.imkerpedia.nl/wiki/index.php/Honingbijen.

The Asian honey bee (Apis cerana)

The Asian honey bee is approximately 10 mm long and has a forewing length of between 7.4 mm – 9.0 mm in length. Asian honey bee could be mistaken for the European honey bee; however, there are few distinguishing features which can be observed:
1) AHB fly very quickly and erratically while feeding on floral resources, while EHB forage much more slowly and methodically;
2) The thorax and abdomen of AHB have less hair than those of EHB;
3) AHB have more prominent, evenly spaced and consistent abdominal striping, compared with EHB that tend to have uneven abdominal striping. EHB generally have thicker black stripes towards the back of abdomen, making the abdomen appear more yellow at the front and darker at the end.

For images on diagnostic features, click the following PADIL link

**Figure 54.** Asian honey bee worker bees. Source: Denis Anderson, CSIRO.

**The European honey bee (Apis mellifera)**

The European honey bee is generally golden/brown with black bands on the abdomen, and has a furry appearance. The European honey bee is approximately 10 - 15 mm long and has a forewing length of between 8.0 mm – 9.7 mm in length.

PADIL link for European honey bee is http://www.padil.gov.au/pests-and-diseases/Pest/Main/135540
Figure 55. European honey bee worker bees, with the queen bee in the centre. Source: Denis Anderson, CSIRO.

Figure 56. Comparison between a foraging Asian honey bee (left) and a European honey bee (right). Source: Paul Zborowski, DAFF Queensland.
A simple key to the workers of the genus *Apis*

This key has been taken from Oldroyd and Wongsiri (2006).

1. Forewing Length
   a) Greater than 12 mm    Giant species (2)
   b) 7 – 10 mm    Cavity nesting species (3)
   c) Less than 7 mm    Dwarf species (4)

2. a) Dorsal surface of the thorax completely covered in tawny yellow hairs
   *A. laboriosa* (India, Nepal, China and Vietnam)
   b) Hairs on the thorax, except on the margins, dark
   *A. dorsata* (distributed widely throughout tropical Asia)

3. a) Hind leg totally black
   b) Hind leg totally yellow
   c) Proximal segments (trochanter, femur) black, distal segments (tibia, tarsus) lighter, tending to yellow
   *A. nuluensis* (mountains, Borneo)

4. a) Posterior tergites of the abdomen have yellow-rufous bands
   *A. florea* (mainland tropical Asia, Middle East)
   b) Posterior tergites of the abdomen black and grey
   *A. andreniformis* (SE Asia, Philippines, Indonesia)

5. a) Forewing length greater than 9 mm
   *A. mellifera* (domesticated import from Europe)
   b) Forewing length less than 9 mm
   *A. cerana* (widely distributed throughout Asia)

6. a) Abdominal tergites yellow
   *A. nigrocincta* (Sulawesi, southern islands of the Philippines)
   b) Abdominal tergites red to rufous
   *A. koschevnikovi* (Malay Peninsular and Borneo)
A simple key to the parasitic Mesostigmata mites of Asian honey bees

This key has been taken from Oldroyd and Wongsiri (2006).

1. a) Body considerably longer than it is wide
   Genus *Tropilaelaps* (parasites of Giant bees and *A. mellifera*)
   b) Body broadly elliptical, as wide or wider than it is long, anal shield triangular and small
   Genus *Varroa* (parasites of cavity nesting bees)
   c) Body broadly pear shaped, approximately the same width and length, anal shield squarish and large
   Genus *Euvarroa* (parasites of dwarf bees)

2. a) Anal plate of adult female mite, horseshoe shaped
   *Tropilaelaps clareae* (parasite of *A. dorsata, A. laboriosa* and *A. mellifera* in Asia)
   b) Anal plate of adult female pear shaped
   *Tropilaelaps koenigerum* (parasite of *A. dorsata* and *A. laboriosa*)

3. a) Peritremes very long, looping up from ventral side, extending beyond the lateral margin and thus visible from the dorsal surface
   *Varroa rindereri* (parasite of *A. koschevnikovi*)
   b) Peritremes shorter, not extending beyond the lateral margin

4. a) Setae of the lateral margin long and slender
   *Varroa underwoodi* (parasite of *A. dorsata* and *A. laboriosa*)
   b) Setae shorter and stout

5. a) Body size ratio (width to length) 1.2-1.3:1
   *Varroa jacobsoni* (parasite of *A. cerana* in Indonesia and SE Asia, also *A. mellifera* in PNG)
   b) Body size ratio ≥ 1.4:1
   *Varroa destructor* (parasite of *A. cerana* in mainland Asia and *A. mellifera* worldwide)
6. a) Body pear-shaped, rounded posteriorly; 39-40 lanceolate setae on the posterior margin
   
   *Euvarroa sinhai* (parasite of *A. florea*)

b) Body more triangular, wider posteriorly, with 47-54 lanceolate setae on the posterior margin
   
   *Euvarroa wongsiri* (parasite of *A. andreniformis*)

*Please note:* the size differential between *V. jacobsoni* and *V. destructor* is not reliably diagnostic and should be confirmed by sequencing the mitochondria (Anderson and Trueman 2000).

**Reference specimens**

Reference specimens held by each state and territory are listed in Table 3 below. These may be useful to assist in the identification of suspected bee pests and pest bees.
<table>
<thead>
<tr>
<th>State / Territory</th>
<th>Facility</th>
<th>Address/Contact details</th>
<th>Reference specimens available</th>
</tr>
</thead>
</table>
| ACT              | Australian National Insect Collection, CSIRO Ecosystem Sciences - Black Mountain Laboratories | Clunies Ross Street, Acton ACT 2601. Phone: 1300 363 400 or (02) 9545 2176 Fax: 61 3 9545 2175 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mite (*Tropilaelaps clareae*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Braula fly (*Braula coeca*)  
• Asian honey bee (*Apis cerana*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
| ACT              | Operational Sciences Program (OSP), ANIC Collection – Canberra Commonwealth Department of Agriculture | Clunies Ross Street, Acton ACT 2601. Phone: 1300 363 400 or (02) 9545 2176 Fax: (03) 9545 2175 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Asian honey bee (*Apis cerana*)  
• Dwarf honey bee (*Apis florea*) |
| VIC              | DPI Victoria – Centre for AgriBioscience | 5 Ring Road, La Trobe University Bundoora VIC 3083 Phone: (03) 9479 5246 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mite (*Tropilaelaps clareae*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Tracheal mite (*Acarapis woodi*)  
• Braula fly (*Braula coeca*) |
| VIC              | Operational Sciences Program (OSP), Melbourne Airport Commonwealth Department of Agriculture | Street address: c/- Customs/AQIS House, Corner Grant and Centre Road, Melbourne Airport VIC 3045 Postal address: PO BOX 1006 Tullamarine VIC 3043 Phone: (03) 8318 6700 Fax: (03) 8318 6701 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mite (*Tropilaelaps clareae*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Tracheal mite (*Acarapis woodi*)  
• Braula fly (*Braula coeca*)  
• Asian honey bee (*Apis cerana*)  
• Giant honey bee (*Apis dorsata*)  
• Dwarf honey bee (*Apis florea*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
| VIC | Museum Victoria | Museum Victoria  
11 Nicholson Street  
Melbourne, Victoria  
Phone: 1300 130 152 | • Asian honey bee (*Apis cerana*)  
• Giant honey bee (*Apis dorsata*)  
• Dwarf honey bee (*Apis florea*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
|---|---|---|
| NSW | DPI New South Wales – Orange Agricultural Institute  
Forest Road  
Orange NSW 2800  
Phone: (02) 6391 3800  
Fax: (02) 62391 3899 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mites (*Tropilaelaps clareae*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Tracheal mite (*Acarapis woodi*)  
• Braula fly (*Braula coeca*)  
• Asian honey bee (*Apis cerana*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
| NSW | Operational Sciences Program (OSP), Sydney  
Commonwealth  
Department of Agriculture  
Street address: 1 Crewe Place,  
Roseberry NSW 2018  
Postal address: PO BOX 657 Mascot NSW 1460  
Phone: (02) 8334 7444  
Fax: (02) 8334 7555 | • Varroa mite (*Varroa destructor*)  
• Varroa mite (*Varroa jacobsoni*)  
• Tropilaelaps mite (*Tropilaelaps clareae*)  
• Tropilaelaps mite (*Tropilaelaps mercedesae*)  
• Tracheal mite (*Acarapis woodi*)  
• Asian honey bee (*Apis cerana*)  
• Giant honey bee (*Apis dorsata*)  
• Dwarf honey bee (*Apis florea*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
| QLD | Queensland Museum  
PO BOX 3300  
South Brisbane 4101  
Phone: (07) 3840 7701  
Fax: (07) 3846 1226 | • Varroa mite (*Varroa destructor*)  
• Braula fly (*Braula coeca*)  
• Giant honey bee (*Apis dorsata*)  
• Bumblebee (*Bombus terrestris*)  
• Small hive beetle (*Aethina tumida*) |
<table>
<thead>
<tr>
<th>State</th>
<th>Address</th>
<th>Contact Information</th>
<th>Insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>QLD</td>
<td>Operational Sciences Program (OSP), Brisbane Commonwealth Department of Agriculture</td>
<td>Street address: 42-44 Qantas Drive, Eagle Farm QLD 4009 Postal address: PO BOX 222 Hamilton QLD 4007 Phone: (07) 3246 8755 Fax: (07) 3246 8639</td>
<td>• Varroa mite (<em>Varroa destructor</em>) • Varroa mite (<em>Varroa jacobsoni</em>) • Tropilaelaps mite (<em>Tropilaelaps clareae</em>) • Tropilaelaps mite (<em>Tropilaelaps mercedesae</em>) • Tracheal mite (<em>Acarapis woodi</em>) • Braula fly (<em>Braula coeca</em>) • Asian honey bee (<em>Apis cerana</em>) • Giant honey bee (<em>Apis dorsata</em>) • Dwarf honey bee (<em>Apis florea</em>) • Bumblebee (<em>Bombus terrestris</em>) • Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
<tr>
<td>QLD</td>
<td>Northern Australian Quarantine Strategy (NAQS), Cairns Commonwealth Department of Agriculture</td>
<td>Street address: Building 114, Catalina Crescent, Airport Business Park, Cairns Airport, Cairns QLD 4870 Postal address: PO BOX 96 AAC Building, Cairns International Airport QLD 4870 Phone: (07) 4241 7800 Fax: (07) 4241 7843</td>
<td>• Varroa mite (<em>Varroa destructor</em>) • Varroa mite (<em>Varroa jacobsoni</em>) • Tropilaelaps mite (<em>Tropilaelaps mercedesae</em>) • Asian honey bee (<em>Apis cerana</em>) • Giant honey bee (<em>Apis dorsata</em>) • Bumblebee (<em>Bombus terrestris</em>)</td>
</tr>
<tr>
<td>QLD</td>
<td>Operational Sciences Program (OSP), Cairns Commonwealth Department of Agriculture</td>
<td>Street address: Building 114, Catalina Crescent, Airport Business Park, Cairns Airport, Cairns QLD 4870 Postal address: PO BOX 96 AAC Building, Cairns International Airport QLD 4870 Phone: (07) 4241 7800 Fax: (07) 4241 7843</td>
<td>• Asian honey bee (<em>Apis cerana</em>) • Giant honey bee (<em>Apis dorsata</em>) • Dwarf honey bee (<em>Apis florea</em>) • Bumblebee (<em>Bombus terrestris</em>)</td>
</tr>
<tr>
<td>NT</td>
<td>Northern Territory Economic Insect Reference Collection (NTEIRC)</td>
<td>Berrimah Farm, Makagon Road, Berrimah, NT 0828. Phone: 61 8 8999 5511 Fax: 61 8 8999 2010</td>
<td>• Varroa mite (<em>Varroa destructor</em>) • Tropilaelaps mite (<em>Tropilaelaps clareae</em>) • Tracheal mite (<em>Acarapis woodi</em>) • Braula fly (<em>Braula coeca</em>) • Asian honey bee (<em>Apis cerana</em>) • Giant honey bee (<em>Apis dorsata</em>) • Bumblebee (<em>Bombus terrestris</em>) • Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
<tr>
<td>State</td>
<td>Location</td>
<td>Contact Information</td>
<td>Insects</td>
</tr>
<tr>
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<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td>NT</td>
<td>Northern Australian Quarantine Strategy (NAQS) and Operational Sciences Program (OSP), Darwin Commonwealth Department of Agriculture</td>
<td>Street address: 1 Pederson Road (Cnr Henry Wrigley Rd), Eaton (Marrara) NT 0812 &lt;br&gt;Postal address: PO BOX 37846 Winnellie NT 0821 &lt;br&gt;Phone: (08) 8998 4900 &lt;br&gt;Fax: (08) 8998 4911</td>
<td>• Varroa mite (<em>Varroa destructor</em>) &lt;br&gt;• Varroa mite (<em>Varroa jacobsoni</em>) &lt;br&gt;• Tropilaelaps mite (<em>Tropilaelaps mercedesae</em>) &lt;br&gt;• Tracheal mite (<em>Acarapis woodi</em>) &lt;br&gt;• Asian honey bee (<em>Apis cerana</em>) &lt;br&gt;• Giant honey bee (<em>Apis dorsata</em>) &lt;br&gt;• Dwarf honey bee (<em>Apis florea</em>) &lt;br&gt;• Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
<tr>
<td>SA</td>
<td>Waite Insect and Nematode Collection (WINC)</td>
<td>Waite Insect and Nematode Collection &lt;br&gt;Waite Research Precinct &lt;br&gt;Urrbrae, South Australia &lt;br&gt;Phone: 61 8 8303 7277 &lt;br&gt;Fax: 61 8 8379 4095</td>
<td>• Asian honey bee (<em>Apis cerana</em>) &lt;br&gt;• Giant honey bee (<em>Apis dorsata</em>) &lt;br&gt;• Bumblebee (<em>Bombus terrestris</em>) &lt;br&gt;• Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
<tr>
<td>TAS</td>
<td>Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE)</td>
<td>Tasmanian DPIPWE &lt;br&gt;13 St Johns Avenue, New Town &lt;br&gt;Tasmania 7008 &lt;br&gt;Phone: 1300 368 550 &lt;br&gt;(Please note: Duplicate collection held at the Launceston DPIPWE offices)</td>
<td>• Varroa mite (<em>Varroa destructor</em>) &lt;br&gt;• Varroa mite (<em>Varroa jacobsoni</em>) &lt;br&gt;• Tropilaelaps mite (<em>Tropilaelaps clareae</em>) &lt;br&gt;• Tracheal mite (<em>Acarapis woodi</em>) &lt;br&gt;• Braula fly (<em>Braula coeca</em>) &lt;br&gt;• Asian honey bee (<em>Apis cerana</em>) &lt;br&gt;• Bumblebee (<em>Bombus terrestris</em>) &lt;br&gt;• Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
<tr>
<td>WA</td>
<td>Invertebrate Collection Database (ICDB), DAFWA</td>
<td>3 Baron-Hay Court, South Perth WA 6151 &lt;br&gt;Phone: (08) 9368 3333</td>
<td>• Braula fly (<em>Braula coeca</em>) &lt;br&gt;• Asian honey bee (<em>Apis cerana</em>) &lt;br&gt;• Giant honey bee (<em>Apis dorsata</em>) &lt;br&gt;• Dwarf honey bee (<em>Apis florea</em>) &lt;br&gt;• Bumblebee (<em>Bombus terrestris</em>) &lt;br&gt;• Small hive beetle (<em>Aethina tumida</em>)</td>
</tr>
</tbody>
</table>
| WA                                | Operational Sciences Program (OSP), Perth | Street address: 9 Fricker Road, Perth airport WA 6105 | • Varroa mite (*Varroa destructor*)
• Varroa mite (*Varroa jacobsoni*)
• Tropilaelaps mite (*Tropilaelaps clareae*)
• Asian honey bee (*Apis cerana*)
• Giant honey bee (*Apis dorsata*)
• Dwarf honey bee (*Apis florea*)
• Bumblebee (*Bombus terrestris*)
• Small hive beetle (*Aethina tumida*) |
| WA                                | Northern Australian Quarantine Strategy (NAQS), Broome | Street address: 401 Port Drive, Broome WA 6725 | • Varroa mite (*Varroa destructor*)
• Varroa mite (*Varroa jacobsoni*)
• Tropilaelaps mite (*Tropilaelaps mercedesae*)
• Asian honey bee (*Apis cerana*)
• Giant honey bee (*Apis dorsata*)
• Bumblebee (*Bombus terrestris*)
• Small hive beetle (*Aethina tumida*) |
| PADIL                             | Pest and Disease Image Library (PADIL) (Images only) | www.padil.gov.au | • Varroa mite (*Varroa destructor*)
• Varroa mite (*Varroa jacobsoni*)
• Tropilaelaps mite (*Tropilaelaps clareae*)
• Tropilaelaps mite (*Tropilaelaps mercedesae*)
• Tracheal mite (*Acarapis woodi*)
• Braula fly (*Braula coeca*)
• Asian honey bee (*Apis cerana*)
• Giant honey bee (*Apis dorsata*)
• Dwarf honey bee (*Apis florea*)
• Bumblebee (*Bombus terrestris*)
• Small hive beetle (*Aethina tumida*) |

**Please Note:** There is a possibility that some reference collections for *Tropilaelaps clareae* are more likely to be *T. mercedesae*, since the former has been split into two species. Collection data would help identify which *Tropilaelaps* mite it is; the true *T. clareae* are from the Philippines and Sulawesi. However, they could also be another of the species of *Tropilaelaps* (*T. thaii* and *T. koenigerum*). For more information about *Tropilaelaps* taxonomy see DL Anderson and MJ Morgan (2007) Genetic and morphological variation of bee-parasitic *Tropilaelaps* mites (*Acari: Laelapidae*): new and redefined species. *Journal of Experimental and Applied Acarology* 43, 1-24.
8 Detection of a suspect exotic pest

8.1 How to report a suspect exotic pest

Early reporting of exotic pests enhances the chance of effective control and eradication. Suspect exotic pests are to be reported immediately to the relevant state/territory agriculture agency by contacting them directly or through the Exotic Plant Pest Hotline (1800 084 881). It should be noted that in some states and territories, the Exotic Plant Pest Hotline only operates during business hours. Where this is the case and calls are made out of hours, callers should leave a message including their contact details, and staff from the department will return the call the following business day.

For Jurisdictional Coordinators of the NBPSP, notification can occur directly to the Chief Plant Health Manager (CPHM) in the relevant State/Territory. The State/Territory Chief Plant Health Manager will then inform the Australian Chief Plant Protection Office (ACPPO) which will notify other relevant Australian Government Departments, relevant state/territory agencies and industry representatives (see Figure 57 below). A diagnostic expert in the relevant jurisdiction should also be contacted to confirm diagnosis.

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**Figure 57. Suspect exotic pest detection reporting flowchart**
8.2 Collection and transport of suspect pest samples

This procedure covers the transport of suspect exotic pest samples, including methods for killing, preservation, packaging and labelling. The methods have been adapted from the Standard operating procedure for the Collection and transport of Emergency Plant Pests, which is a supporting document of PLANTPLAN (Plant Health Australia, 2013).

Correct identification of suspect exotic pests is central to effective control of pests and for the detection of new Emergency Plant Pests. The collection and transport of suspect exotic pests presents a potential risk of escape of exotic pests to the environment. The following procedure is intended to minimise this risk.

Different pests require different handling techniques depending on the requirements for preservation and of the laboratory receiving the sample for confirmatory diagnostics. Therefore it is best to contact the diagnostic laboratory to determine their preference for sample preservation.

8.2.1 Selection of laboratory and confirmation of mailing arrangements

- The CPHM will select the preferred laboratory and scientist for sample diagnosis.
- If the laboratory is interstate, it will be necessary to seek appropriate permits from interstate CPHMs.
- The quarantine officer or CPHM will confirm with the Manager of the Diagnostic Laboratory that they are prepared to accept the sample(s). He/she will also confirm the mailing address and arrangements for consignment and receipt of samples, including packaging requirements.

8.2.2 General principles for collecting samples

- Complete a sample submission form at time of sampling (include details such as host, plant parts affected, location (GPS coordinates), date of sampling, property owner, contact details and any other relevant information). Hold a copy of details with a duplicate sample.
- Disinfect implements (eg with 80% v/v ethanol or 0.5% v/v available chlorine solution, as appropriate) prior to and after each sampling.
- It is essential that the time between sampling and dispatch of the sample for identification be kept to a minimum. Do not send samples on a Friday unless first consulting with receiving laboratory.
- Appropriate hygiene protocols for collecting samples and disinfecting hands, footwear and clothing should be followed.

8.2.3 Killing and preserving samples

In most instances insect specimens should be sent dead and preserved in a manner required by the laboratory.
• **Do not send live insects.** In exceptional circumstances, the diagnostic laboratory may require live specimens. In such cases, special arrangements must be made, ensuring secure transportation, prompt collection of samples etc. These circumstances will be decided by the State Coordination Centre and/or CPHM.

• The majority of pest samples will already be dead upon examination (e.g. samples on sticky mats or found after alcohol washing etc), however if not then the correct procedure should be followed for specimen killing and preservation, so that its identity can be accurately confirmed by a specialist diagnostician. Check with the diagnostic laboratory to determine their preference for sample preservation. In general, suspect pests should be killed by freezing or immersing in 80% ethyl alcohol (e.g. methylated spirits) for 24 hrs prior to sending.

### 8.2.4 How to package samples

• Place the specimen(s) in a plastic or glass vial or small jar, or in a crush-proof box with tissues.

• Include loosely crumpled facial tissues or similar in the bottom of containers to help prevent damage to fragile insects and absorb any free fluids.

• Tape the lid securely to avoid accidental spillage. Seal receptacle with a tamperproof seal.

• Clearly label all samples (refer to Labelling Samples section below).

• Place the vial, jar or box containing the sample into a press seal bag with some absorbent material such as paper towelling to absorb any accidental spillage that may occur in transit.

• Triple bag the sample, disinfecting between layers with the exterior bag being durable. All paper work must be placed in a separate plastic bag and enclosed between the second and third (outer) layer of the triple packaging.

• Where a Chain of Evidence is required the bag should be sealed with evidence tape.

• Alert the receiving laboratory of expected arrival of insect samples.

### 8.2.5 Sample labelling instructions

• Label each sample clearly using an alcohol-proof marker

• Key list the samples and label each clearly

• Secure labels to the outside (and if appropriate to the inside) of the sample bag or container. A label should also be included in the bag in case the outer label is destroyed.
8.2.6 General requirements for packaging samples for surface and air transport

- Include a covering note to the diagnostic facility outlining that the sample is a suspect exotic pest, and if possible, indicate what you suspect the pest to be.
- Include the sample submission form in a separate plastic bag.
- Label the package with:
  - The recipient’s name, address and telephone number
  - The sender’s name, address and telephone number
  - “Urgent – Diagnostic sample. Keep cool”
- Pack the samples securely using the following procedures:
  - Place the sealed bag/envelope (containing the sample) into a plastic screw-top, rubber sealed container (e.g. Bio-bottles) and then into a small sturdy box (i.e. made out of rigid corrugated cardboard, tin or light wood).
  - Fill the remaining space in the box with at least 100 mm of padding (foam chips, crumpled paper, bubble wrap etc) to prevent the sample from moving about inside the box during transit. Ensure the lid is secured.
  - Wrap the box securely in packing paper.

8.2.7 Dispatching samples interstate

- Check if there are any interstate quarantine regulations that need to be complied with should a sample be sent interstate. ENSURE APPROPRIATE PERMITS ARE OBTAINED.
- Notify the diagnostian that a suspect emergency plant pest is being sent to the laboratory and the estimated time of arrival. The diagnostian should be informed of any additional precautions that may be required on arrival e.g. open sample in appropriate containment facilities.
- Choose the most reliable and fastest method of dispatching the sample.
- If you expect a delay of more than 2 days in sending samples, store sample under appropriate conditions prior to sending.
- Ensure samples are sent directly to the chosen laboratory.
- Attach consignment notice to outside of package.

Samples must either be dispatched to the diagnostic facility by a courier provider which ensures overnight or same day delivery of package. On arrival signature receipt is required. Alternately, the sample can be hand delivered to the diagnostic facility. Remember to keep samples cool and out of direct sunlight.
9 Purchase of chemicals and supplies used in the NBPS

Sticky mats, chemical strips (Apistan and Bayvarol) and Apithor harbourages are housed and distributed by PHA. Apistan or Bayvarol miticide strips are used with sticky mats in sentinel hives for detection of exotic mites (Varroa sp. and Tropilaelaps sp.). Apithor harbourages are used in hives for detection of SHB. The current suppliers of these products are listed below along with the ordering process.

9.1 Apistan and Bayvarol strips

The current supplier of Apistan strips is Ceracell Beekeeping Supplies Ltd. The current supplier of Bayvarol strips is Ecroyd Beekeeping Supplies. Both companies are in New Zealand. Contact details are provided in Table 4 below.

9.1.1 Ordering procedure

1. Apistan strips can be ordered through the Ceracell website and Bayvarol strips can be ordered through the Ecroyd’s website. Contact Ceracell or Ecroyd and get a quote and information on availability. The availability is necessary to obtain as the expected delivery date is required on the Australian Pesticides and Veterinary Medicines Authority (APVMA) application for the Consent to Import an Unregistered Product (see below).

2. Prior to ordering the strips, an “APPLICATION FOR CONSENT TO IMPORT UNAPPROVED ACTIVE CONSTITUENTS OR UNREGISTERED AGRICULTURAL OR VETERINARY CHEMICAL PRODUCTS TO BE SUPPLIED AND/OR USED IN ACCORDANCE WITH AN APVMA PERMIT” must be completed and submitted to the APVMA. The form is available at the APVMA website: www.apvma.gov.au/supply/import.php#forms and a copy is provided in Appendix 2, page 106.

3. As the Department of Agriculture is the holder of the Minor Use Permit (Permit 14167, Appendix 3, page 108), they will need to submit the “Consent to Import” form to the APVMA, by faxing or emailing to importconsent@apvma.gov.au. PHA will assist in preparing information required. If further information is required, contact Import Consents by phone (02 6210 4793) or fax (02 6210 4741).

4. When the “Consent to Import” is issued, the strips can be ordered. Usually two cartons of Bayvarol strips (800 each, 1600 in total) and 2000 Apistan strips (packs of 10) are sufficient for approximately 2-3 years supply.

5. Strips are received by PHA and sent to Jurisdictional Coordinators. Packaging in a cardboard box is sufficient to protect the strips from damage during transit.

6. Strips must be used under permit requirements (see Appendix 3, page 108). Read the label and MSDS prior to use (see Appendix 5, page 118).
<table>
<thead>
<tr>
<th>Product</th>
<th>Supplier</th>
<th>Address</th>
<th>Contact details</th>
<th>Product information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apistan</td>
<td>Ceracell Beekeeping Supplies Ltd.</td>
<td><strong>Street address:</strong> 24 Andromeda Crescent East Tamaki 2013 Auckland, New Zealand  <strong>Postal address:</strong> PO Box 204184 Highbrook, Manukau Auckland New Zealand 2161</td>
<td>Phone: +64 9 274 7236, Fax: +64 9 274 0368 Email: <a href="mailto:info@ceracell.co.nz">info@ceracell.co.nz</a> Website: <a href="http://www.nzbeekeepingsupplies.co.nz/go/contact_us">www.nzbeekeepingsupplies.co.nz/go/contact_us</a></td>
<td>Product code: VAR3750 Cost: Price per strip is $2.77 plus GST for order of 1000+. Strips are sold in sealed pouches of 10.</td>
</tr>
<tr>
<td>Bayvarol Ecroyd Beekeeping Supplies</td>
<td><strong>Street address:</strong> 6A Sheffield Crescent Burnside Christchurch, New Zealand 8053  <strong>Postal address:</strong> PO Box 5056 Papanui, Christchurch New Zealand 8542</td>
<td>Phone: +64 3 358 7498 Fax: +64 3 358 8789 Email: <a href="mailto:ecroyd@beehealthy.co.nz">ecroyd@beehealthy.co.nz</a> Website: <a href="http://www.ecroyd.com/Home">www.ecroyd.com/Home</a></td>
<td>Cost: The cost varies per strip depending on the amount ordered. If ordering a carton of 800 strips, the cost is $1.40 per strip, amounting to $1120 in total.</td>
<td></td>
</tr>
</tbody>
</table>
9.2 Sticky mats

Sticky mats/boards can be ordered from Starkeys or Permark. The NBPSP currently uses the Permark sticky mats as they are considerably cheaper.

9.2.1 Starkeys

Starkeys high quality Glue Pads can be ordered through the website www.starkeys.com.au or the contact details below.

Street address:
46 Achievement Way
Wangara WA 6065

Postal address:
PO BOX 1349
Wangara 6947

Phone: +61 (0) 8 9302 2088
Fax: +61 (0) 8 9302 2138

Product name: Starkeys Genuine Glue Boards

9.2.2 Permark

The white cardboard sticky mats can be ordered through http://www.permark.com.au/ or by contacting Amrat Parbhu on (02) 9911 6656.

Details of the sticky mats are below:

Beemite Sticky Boards (see Figure 58)
Size: 300 x 460mm irregular shape
Material: 325gsm white board
Adhesive: 3M permanent adhesive on the rear
Cost: The cost per sticky board is $2.46 for orders 2000+
Figure 58. PHA designed sticky mat used in the NBPSP
9.3 Apithor harbourage

Apithor harbourages can be ordered through the Apithor website (https://apithor.com.au/sales.html) or by contacting Ensystex through the details below.

Phone: 13 35 36
Email: contact@apithor.com.au

Prices depend on the amount ordered, with 20 Units costing a total of $1980 + GST.

Apithor is now registered for use in all states and territories of Australia (see Appendix 3, page 115). Refer to the product label (page 121) and MSDS (page 133) prior to use.

10 Maintenance of APVMA permits

10.1 Minor use permit for Bayvarol and Apistan

Bayvarol and Apistan are used in the NBPSP under an APVMA minor use permit (14167, see Appendix 3, page 108). The permit holder is the Department of Agriculture. Permit requirements include:

- Suspend strips in spaces between the combs in the central brood rearing area for 24-48 hrs.
- Do not suspend strips in honey supers.
- Do not apply strips more frequently than every 6-8 weeks.
- Do not apply more than 9 times per annum in any one apiary.
- The strips should not be used during peak honey flow as residues may result in the wax of the honey comb. If Strips are used during honey flow then comb honey should not be consumed.
- Honey from treated hives cannot be sold, supplied or otherwise made available for human consumption until or unless the residues are at or below the following MRLs: Fluvalinate MRL ≤ 0.01 mg/kg in honey, Flumethrin MRL ≤ 0.005 mg/kg in honey. The procedure for MRL testing is provided in section 7.1.1.3.
- Product labels and MSDS (provided in Appendix 5, page 118) should always be read prior to use.

The minor use permit for Apistan and Bayvarol expires on 30 September 2015. Before the permit expires, an application for renewal of this permit (Category 20 form) will need to be submitted to the APVMA by The Department of Agriculture. Go to the APVMA website www.apvma.gov.au/permits/apply/index.php to download the most current Category 20 form. Note that new legislation will come into effect on July 1 2014 that is likely to change the process for renewing permits.
10.2 Emergency use permit for Bayvarol and Apistan

If an exotic mite (e.g. Varroa) enters Australia, then Apistan and Bayvarol will be used under the emergency use permit (11761, see Appendix 3, page 111). The permit holder is the Department of Agriculture. Permit requirements are similar to the minor use permit and include:

- For use in hives within a 25 km radius of the location of an identified incursion of Varroa or Tropilaelaps mites or their exotic bee hosts.
- Suspend strips between combs in the central brood rearing area for a period of 1 to 14 days. The period should be determined based on suspected mite population levels. Lower population levels may be detected the longer strips are kept in place.
- DO NOT suspend strips in honey supers.
- SURVEILLANCE MONITORING: DO NOT apply strips more frequently than every 6-8 weeks. DO NOT apply more than 9 times per year in any one apiary.
- INCURSION MONITORING: At the start of the surveillance program, treat selected hives a maximum of every week for 4-6 weeks, then every 6 weeks for 6 months, then every 2-3 months for the following 12 months.
- Ideally, the strips should not be used during peak honey flow as residues may result in the wax of the honey comb.
- If monitoring is conducted over a significant period of time, a minimum of 2 frames from each brood box should be replaced annually to minimise build-up of residues in wax.
- Product labels and MSDS (provided in Appendix 5, page 118) should always be read prior to use.

The emergency use permit expires on 30 September 2015. Before the permit expires, an application for renewal of this permit (Category 22 form) will need to be submitted to the APVMA by the Department of Agriculture. Go to the APVMA website www.apvma.gov.au/permits/apply/index.php to download the most current Category 22 form. Note that new legislation will come into effect on July 1 2014 that is likely to change the process for renewing permits.

Emergency use stocks of Apistan and Bayvarol are housed by the Department of Agriculture. They are located in the Locked storeroom in the Australian Plague Locust Commission (APLC) secure vehicle cage. The address is:

Unit 7, 50 Collie Street
Fyshwick, ACT, 2609

For access to these stocks, phone the following numbers in order until contact is made and arrangements agreed for access to these stocks.

0428 264 083 – Chris Adriaansen
0428 329 414 – Walter Spratt
0478 489 443 – Larry Veness
Access to the APLC secure vehicle cage and storeroom can only be gained by an APLC officer. The Department of Agriculture officers do not have security clearance to enter these areas unless accompanied by an APLC officer.

The following procedure should be followed:

1. Use of any of these stocks is permitted only for emergency use (suspected or confirmed incursion)
2. Make contact with APLC by phoning the above phone numbers (in order) until person-to-person contact is made with an officer and arrangements are agreed for access to these stocks.
3. Meet the APLC officer as arranged at APLC Fyshwick (address above) and remove stock required.
4. Distribute and use stocks in accordance with permit conditions, ensuring that a valid permit and Material Safety Data Sheet/s (MSDS) are provided with all stocks distributed (see Appendix 5, pages 119 and 128 for MSDS).
5. Ensure proper records of distribution and application are kept.

11 Data entry into workbooks and NBPS interface

Data is entered by jurisdictional coordinators into an excel workbook and submitted to PHA every two months following each surveillance run. Each file is saved in a separate folder for each jurisdiction and named according to month and year of testing. The instructions for coordinators on how to enter data into the excel workbook is contained within the workbook. There are separate tabs within the workbook to record the sentinel hive surveillance with sticky mat and acaricide strips (NBPS tab), results from Tracheal mite analysis by Bugs for Bugs (BUGS tab), additional surveillance including sugar shaking, drone uncapping, alcohol washing, swarm capture, remote surveillance hives, catchboxes, log traps, floral sweep netting, additional/ad-hoc SHB surveillance conducted on hives that are not sentinel hives, as well as additional/ad-hoc Tracheal mite surveillance are recorded in the (BSUR tab) and MRL testing (MRLP tab). Small hive beetle (SHB) surveillance conducted on sentinel hives is recorded in a different excel workbook (SHBP workbook) as not all jurisdictions conduct this surveillance.

The NBPS online interface is located at http://nbpsp.planthealthaustralia.com.au. Data is submitted into the online interface by PHA. PHA as administrators of the program are the only agency with access to this database. To obtain a new username (PHA administrators only), email or phone the database administrator (currently Rachel Gordon) through the contact details listed on the home page (email Rachel@ausvet.com.au, phone 02 6362 6447).
The procedure for uploading data into the online interface and retrieving reports is described below:

- Log onto the online interface.
- Go to “Submit” tab and select “Submit data”.
- Enter data separately for each state/territory and for each project/tab within the workbook. Select the project from the appropriate drop down box and click “Select report to submit”. Summaries of each of the possible drop down boxes include:
  - Bee Surveillance (BSUR) – This includes data submission for other bee surveillance activities, including alcohol washing, drone uncapping, sugar shaking, catchboxes, remote surveillance hives, log traps, swarm capture at ports and floral sweep netting. Tracheal mite and Small hive beetle surveillance that is conducted on an ad-hoc basis (i.e. not linked to a sentinel hive with an ID code) can also be entered into this workbook.
  - National Bee Pest Surveillance (NBPS) – This includes submission of sticky mat and acaricide results directly relevant to sentinel hives that have been assigned an identification number.
  - Bugs for Bugs (BUGS) – This includes data relevant to Tracheal mite diagnostics undertaken by Bugs for Bugs relating to specific sentinel hives with identification numbers.
  - Minimum Residue Limit (MRLP) – This relates to the MRL results for honey/wax after testing with acaricides in sentinel hives.
  - Small Hive Beetle Surveillance (SHBS) – This includes submission of Apithor and oil trap results from sentinel hives that have been assigned an identification number.
- Copy the data from the excel workbook, including the two header rows, paste into the box and click submit. For example, if uploading from the NPBS tab, copy in one action the header row labelled NPBS, the row beneath with column headings “site_num, specific, general, hive_status” etc, and the data in the rows beneath. Note that all compulsory fields must be completed or data cannot be uploaded.
- After submitting, uploaded data should be viewed to ensure it all uploaded correctly. Check that the correct number of rows has been inserted.
- Summary reports or detailed project data can be viewed and exported from the online interface. Reports can be generated for each project by state/territory or Australia wide and for defined time periods.
- To view Summary reports, go to “View” tab and select “Summary reports”. Select the project that you want to view reports of from the drop down list e.g. BSUR, NBPS, BUGS etc, click “Select Project” and then click on the summary. The date period can then be altered using the drop down boxes that appear with the summary. Select “Download data” to export.
- To view detailed project data, go to “View” tab and select “Detailed project data”. Select the project that you want to view reports of from the drop down list e.g.
BSUR, NBPS, BUGS etc. Define the time period using the calendar function and select if you want to view data by state/territory or Australia wide. Select “View data” or select “Download data” to export.

- Note that although the majority of Tracheal mite surveillance data is recorded in the BUGS project, some data may have been recorded in the BSUR project, for example, where bees from collected swarms are analysed. Therefore when obtaining summary and/or detailed project data for Tracheal mite surveillance, ensure that both projects (BUGS and BSUR) are interrogated in the online database.

- Note that although the majority of SHB surveillance data is recorded in the SHB surveillance (SHBS) project, some data may have been recorded in the BSUR project, for example, when hives without sentinel hive codes are used for trapping. Therefore when obtaining summary and/or detailed project data for SHB surveillance, ensure that both projects (SHBS and BSUR) are interrogated in the online database.
## 12 Appendix 1 Sentinel hive locations

**Table 5. Locations of sentinel hives included in the NBPSP**

<table>
<thead>
<tr>
<th>State/territory</th>
<th>Name of area</th>
</tr>
</thead>
<tbody>
<tr>
<td>New South Wales</td>
<td>Port Botany</td>
</tr>
<tr>
<td></td>
<td>Newcastle</td>
</tr>
<tr>
<td></td>
<td>Wollongong</td>
</tr>
<tr>
<td></td>
<td>Richmond</td>
</tr>
<tr>
<td></td>
<td>Goodward Island</td>
</tr>
<tr>
<td></td>
<td>Darling Harbour</td>
</tr>
<tr>
<td></td>
<td>Kurnell</td>
</tr>
<tr>
<td></td>
<td>Chifley</td>
</tr>
<tr>
<td></td>
<td>Jervis Bay</td>
</tr>
<tr>
<td>Northern Territory</td>
<td>Darwin</td>
</tr>
<tr>
<td></td>
<td>Darwin Airport</td>
</tr>
<tr>
<td></td>
<td>Berrimah Farm</td>
</tr>
<tr>
<td>Queensland</td>
<td>Brisbane</td>
</tr>
<tr>
<td></td>
<td>Cairns</td>
</tr>
<tr>
<td></td>
<td>Townsville</td>
</tr>
<tr>
<td>South Australia</td>
<td>Port Adelaide</td>
</tr>
<tr>
<td></td>
<td>Port Pirie</td>
</tr>
<tr>
<td></td>
<td>Wallaroo</td>
</tr>
<tr>
<td>Tasmania</td>
<td>Hobart</td>
</tr>
<tr>
<td></td>
<td>Devonport</td>
</tr>
<tr>
<td></td>
<td>Bell Bay</td>
</tr>
<tr>
<td></td>
<td>Burnie</td>
</tr>
<tr>
<td>Victoria</td>
<td>Melbourne</td>
</tr>
<tr>
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<td>Geelong</td>
</tr>
<tr>
<td></td>
<td>Portland</td>
</tr>
<tr>
<td></td>
<td>Westernport</td>
</tr>
<tr>
<td>Western Australia</td>
<td>Fremantle</td>
</tr>
<tr>
<td></td>
<td>Kwinana</td>
</tr>
<tr>
<td></td>
<td>Perth Airport</td>
</tr>
</tbody>
</table>
Figure 59. Locations of sentinel hives included in the NBPSN
13 Appendix 2: Consent to import Bayvarol or Apistan form

The following ‘Consent to import’ form is available at the APVMA website:

APPLICATION FOR CONSENT TO IMPORT UNAPPROVED ACTIVE CONSTITUENTS OR UNREGISTERED AGRICULTURAL OR VETERINARY CHEMICAL PRODUCTS TO BE SUPPLIED AND/OR USED IN ACCORDANCE WITH AN APVMA PERMIT

Prior to submission of this application, applicants must be familiar with the information below.

Applications for Consent to Import must be lodged by a person who is either a resident of or is carrying on a business in Australia.

The Consent to Import issued by the APVMA, is generally only valid for importations during the two-week period either side of the estimated date of importation and only covers the importation specifically applied for. Should there be any changes to the information provided, a new application is required. If unable to estimate an exact date of importation, the month of the expected importation is to be nominated.

In the case of importations associated with an issued APVMA Permit (not Permit 7250), the consent validity is usually a period of 3 months following the issue of the permit. Where multiple shipments are expected, applicants may seek a validity period of 12 months, provided this is within the validity period of the permit.

If the imported chemical is to be used in trials under APVMA Small Scale Permit No. 7250, the applicant is legally bound to abide by the conditions attached to that permit and may also be subject to audit. Details of this permit can be found at http://permits.apvma.gov.au/PER7250.PDF.

Should any condition of Permit 7250 not be able to be met, an application for a specific APVMA Permit is required. Further information and application forms can also be found on the APVMA website at http://www.apvma.gov.au/permits/index.php. The permit number is to be quoted alongside ‘Reason for importation’.

Applicants should be aware that some commodities require additional approvals from agencies other than the APVMA. It is the applicant’s responsibility to check with these agencies. Agencies include:

- Therapeutic Goods Administration
- Office of Chemical Safety and Environmental Health
- Office of the Gene Technology Regulator
- Australian Quarantine and Inspection Service
- Department of Agriculture, Fisheries and Forestry
- Department of Foreign Affairs and Trade
- Department of Sustainability, Environment, Water, Population and Communities

I declare that:
- I have read and understand the information on this page
- the information provided in this form is complete and correct.

I understand that:
- providing false or misleading information is a serious offence.

Name of Applicant: __________________________________________

Organisation Name: _________________________________________

Signature of Applicant: ________________________________ Date: ___________ / __________ / __________

Version 1
APPLICATION FOR CONSENT TO IMPORT UNAPPROVED ACTIVE CONSTITUENTS OR UNREGISTERED AGRICULTURAL OR VETERINARY CHEMICAL PRODUCTS TO BE SUPPLIED AND/OR USED IN ACCORDANCE WITH AN APVMA PERMIT

I wish to apply for a Consent to Import for the following:

<table>
<thead>
<tr>
<th>Name of agvet chemical product:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active constituent(s):</td>
</tr>
<tr>
<td>Manufacturer’s name and address:</td>
</tr>
<tr>
<td>Quantity to be imported:</td>
</tr>
<tr>
<td>Reason for importation: To be dealt with under the conditions of APVMA permit number:</td>
</tr>
</tbody>
</table>

If the reason is for use under Permit 7250, also provide the following required information:

<table>
<thead>
<tr>
<th>Crop/Host Species:</th>
<th>Disease/Pest:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application/Dose Rate:</td>
<td>Application/Dose Frequency:</td>
</tr>
<tr>
<td>Trial size: (e.g. Number of animals treated, number of hectares treated)</td>
<td></td>
</tr>
<tr>
<td>Port of entry:</td>
<td></td>
</tr>
<tr>
<td>Name and address of importing agent:</td>
<td></td>
</tr>
<tr>
<td>Estimated date/period of arrival: For issued permits you may import multiple shipments during a 12 month period</td>
<td></td>
</tr>
</tbody>
</table>

If a subsequent request for 12 months import period under an existing issued permit, detail quantity imported in previous 12 months period.

<table>
<thead>
<tr>
<th>Name of Applicant:</th>
<th>ABN:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of Contact Person:</td>
<td></td>
</tr>
<tr>
<td>Contact Details of Applicant:</td>
<td>Phone:</td>
</tr>
<tr>
<td>E-mail:</td>
<td>Fax:</td>
</tr>
<tr>
<td>Postal address of Applicant:</td>
<td></td>
</tr>
</tbody>
</table>

Signature: Date:

Send application to: Imports Coordinator
Australian Pesticides and Veterinary Medicines Authority
Regulatory Strategy and Compliance Program
PO Box 6182
Kingston ACT 2604
Or Email: importconcera@apvma.gov.au
Or Fax 02 6210 4813

Enquiries: Imports Coordinator
Phone: 02 6210 4793

Further information can be found at:
14 Appendix 3: Chemical Permits

14.1 APVMA Permit for minor use of Bayvarol and Apistan (Permit 14167)

PERMIT FOR SUPPLY AND USE OF AN UNREGISTERED AGVET CHEMICAL PRODUCT

PERMIT NUMBER - PER14167

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a person, as stipulated below, to possess the product for the purposes of supply and to supply the product to a person who can use the product under permit. This permit also allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows a person, as stipulated below, to claim that the product can be used in the manner specified in this permit. If this permit were not issued, supply of the product as specified below would constitute an offence under section 78 of the Agvet Code.

THIS PERMIT IS IN FORCE FROM 1 JULY 2013 TO 30 SEPTEMBER 2015.

Permit Holder:
DEPARTMENT OF AGRICULTURE FISHERIES AND FORESTRY
18 Marcus Clarke
STREET CIVIC ACT
2601

Persons authorised under this permit to supply and make claims:
DEPARTMENT OF AGRICULTURE, FISHERIES & FORESTRY

Persons authorised under this permit to use the product and make claims:
Persons generally who have been authorised by the Secretary of the Department of Agriculture Fisheries and Forestry or by the Chief Plant Protection Officer (or equivalent) as appropriate of the Commonwealth/State/Territory.

Products to be used:
APISTAN VARROA CONTROL FOR BEES
Containing: 824.00 mg/strip FLUVALINATE as the only active constituent.

BAYVAROL STRIPS
Containing: 3.60 mg/strip FLUMETHRIN as the only active constituent.
Directions for Use:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Pest</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIVES OF EUROPEAN HONEYBEES</td>
<td>FOR THE DIAGNOSIS AND SURVEILLANCE OF VARROA MITE AND TROPILAELEPS MITE.</td>
<td>EACH POLYMER MATRIX STRIP CONTAINS 824 mg FLUVALINATE or 3.6 mg FLUMETHRIN. USE A MAXIMUM OF 4 STRIPS PER HIVE.</td>
</tr>
</tbody>
</table>

Critical Use Comments:
- FOR USE IN THE AUSTRALIAN BEE SURVEILLANCE PROGRAM.
- SUSPEND STRIPS IN SPACES BETWEEN THE COMBS IN THE CENTRAL BROOD REARING AREA FOR 24-48 HRS.
- DO NOT SUSPEND STRIPS IN HONEY SUPERS.
- DO NOT APPLY STRIPS MORE FREQUENTLY THAN EVERY 6-8 WEEKS.
- DO NOT APPLY MORE THAN 9 TIMES PER ANNUM IN ANY ONE APIARY.
- THE STRIPS SHOULD NOT BE USED DURING PEAK HONEY FLOW AS RESIDUES MAY RESULT IN THE WAX OF THE HONEY COMB.

Withholding Period:
HONEY - NIL
COMB HONEY - DO NOT MAKE COMB HONEY AVAILABLE FOR HUMAN CONSUMPTION IF HIVES ARE TREATED DURING HONEY FLOW.

First aid instructions:
If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126.

Jurisdiction:
ALL States

CONDITIONS

Supply
(i) Persons authorised by this permit to supply the product must supply the product in a container that complies with the requirements of regulations 18(1) and (2) of the Agricultural and Veterinary Chemicals Code Regulations. Attached to this container must be a label which is identical in content and format to the labels in Attachment 1 and Attachment 2.

Use
(ii) Persons authorised by this permit to use the product for the purposes specified in this permit must read, or have read to them, the permit.

(iii) Unless otherwise specified in this permit, users must comply with the instructions on
the label of the product, particularly those instructions relating to protection, precaution, safety directions, and storage and disposal.

(iv) The permit holder must ensure that honey from treated hives will not be sold, supplied or otherwise made available for human consumption until or unless the residues in such produce are at or below the maximum residue limit (MRL) listed below:

- Fluvalinate MRL T*0.01 mg/kg in honey.
- Flumethrin MRL T*0.005 mg/kg in honey.

(v) The permit holder must maintain a register of the number of strips of product used for each location. This information must be provided to the APVMA on request.

Claims

(vi) Persons authorised by this permit to supply and use the product may claim that the product can be used as specified in this permit.

Issued by

Delegated Officer
Veterinary Medicines Program
14.2 APVMA permit for emergency use of Bayvarol and Apistan (Permit 11761)

PERMIT TO ALLOW EMERGENCY USE AND SUPPLY OF AN UNREGISTERED AGVET CHEMICAL PRODUCT

FOR THE SURVEILLANCE OF SPECIFIED MITES IN EUROPEAN HONEYBEE HIVES

PERMIT NUMBER - PER11761

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a Supplier (as indicated) to possess the product for the purposes of supply and to supply the product to a person who can use the product under permit. This permit also allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows the Permit Holder, the Supplier (if not one and the same) and any person stipulated below to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 1 OCTOBER 2010 TO 30 SEPTEMBER 2015.

Permit Holder and Supplier:

DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY GPO
BOX 858,
CANBERRA CITY, ACT 2601

Persons who can use the product under this permit:

Persons who have been authorised by the Secretary of the Department of Agriculture Fisheries and Forestry or by the Chief Plant Protection Officers (or equivalent) as appropriate of the Commonwealth/State/Territory.
CONDITIONS OF USE

Product to be used:

APISTAN VARROA CONTROL FOR BEES
Containing: 824.00 mg/ea TAU-FLUVALINATE as the only active constituent.

BAYVAROL STRIPS
Containing: 3.60 mg/ea FLUMETHRIN as the only active constituent.

Directions for Use:

<table>
<thead>
<tr>
<th>Animal</th>
<th>Pest</th>
<th>Rate</th>
</tr>
</thead>
</table>
| Hives of European Honeybees | For the diagnosis and surveillance of Varroa and Tropilaeleps mites. | APISTAN: Tau-fluvalinate
Use 2 strips per brood box|
|                         |                                  | BAYVAROL: Flumethrin
Use 4 strips per brood box |

Critical Use Comments:

- For use in hives within a 25 km radius of the location of an identified incursion of Varroa or Tropilaeleps mites or their exotic bee hosts.

- Suspend strips between combs in the central brood rearing area for a period of 1 to 14 days. The period should be determined based on suspected mite population levels. Lower population levels may be detected the longer strips are kept in place.

- DO NOT suspend strips in honey supers

- SURVEILLANCE MONITORING: DO NOT apply strips more frequently than every 6-8 weeks. DO NOT apply more than 9 times per year in any one apiary.

- INCURSION MONITORING: At the start of the surveillance program, treat selected hives a maximum of every week for 4-6 weeks, then every 6 weeks for 6 months, then every 2-3 months for the following 12 months.

- Ideally, the strips should not be used during peak honey flow as residues may result in the wax of the honey comb.

- If monitoring is conducted over a significant period of time, a minimum of 2 frames from each brood box should be replaced annually to minimise build up of residues in wax.
**Withholding Period:**

HONEY – Honey from treated hives must not be made available for human consumption until any residues are at or below the relevant maximum residue limits.

COMB HONEY – DO NOT make comb honey available for human consumption.

**Jurisdiction:**

All States.

**Additional Conditions:**

PERSONS who wish to prepare for use and/or use the products for the purposes specified in this permit must read, or have them read, the permit particularly the information included in DETAILS OF PERMIT and CONDITIONS OF PERMIT.

The users of this permit must comply with the instructions on the approved label of the product, particularly those instructions relating to protection, precaution, safety directions, first aid, storage and disposal.

The supplier must supply the product in a container that complies with the requirements of section 18(1) of the Agricultural and Veterinary Chemicals Code Regulations. Attached to this container must be a label, which is identical in content and format to the label in Attachment 1.

To facilitate domestic trade in produce treated under this permit the APVMA has established the maximum residue limits (MRL) listed below.

- **Fluvalinate**  
  T*0.01mg/kg in honey.
- **Flumethrin**  
  T*0.005mg/kg in honey.

The permit holder must maintain a register for all product usage.

**Issued by**

Delegated Officer
14.3 APVMA renewal forms for minor and emergency use permits

Minor use permits are currently renewed by completing a Category 20 form. An application for a fresh emergency use permit which is identical to an existing emergency use permit must be made under Category 22. Category 20 and 22 forms can be downloaded from the APVMA website www.apvma.gov.au/permits/apply/index.php. Note that new legislation will come into effect on July 1 2014 that is likely to change the process for renewing permits.
15 Appendix 4: Apithor Registration (Product number 66708)

Product: APITHOR HIVE BEETLE HARBOURAGE INSECTICIDE

Status: Registered (2013-12-09)  
Product number: 66708

General details
Registrant: ENSYSTEX AUSTRALASIA PTY LTD
Category: AgChem
Product type: INSECTICIDE

Poison schedule: 0  
Registration date: 2013-12-09

Expiry date: 2014-06-30

Formulation type: SOLID (INC. MOSQUITO COILS AND CANDLES, PREMIXES)

Constituents details

<table>
<thead>
<tr>
<th>Constituent name</th>
<th>Chemical group</th>
<th>Type</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIPRONIL</td>
<td>NITRILE</td>
<td>Active</td>
<td>0.48</td>
<td>g/kg</td>
</tr>
</tbody>
</table>

Pack size details

| Pack size information | #(20x11g) | #220g | *(10x11g) | *110g | 11g | 55g(5X11g) |

State registration details

<table>
<thead>
<tr>
<th>State</th>
<th>Status</th>
<th>First registered date</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
<tr>
<td>NSW</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
<tr>
<td>NT</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
<tr>
<td>QLD</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
<tr>
<td>SA</td>
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</tr>
<tr>
<td>TAS</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
<tr>
<td>VIC</td>
<td>Registered</td>
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</tr>
<tr>
<td>WA</td>
<td>Registered</td>
<td>2013-12-09</td>
</tr>
</tbody>
</table>

Label details

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>54227</td>
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# Protected data details

<table>
<thead>
<tr>
<th>Appl no</th>
<th>Data no</th>
<th>Ref. product</th>
<th>Author</th>
<th>Title</th>
<th>Study date</th>
<th>Data type</th>
<th>End date</th>
<th>Auth party</th>
</tr>
</thead>
<tbody>
<tr>
<td>54227</td>
<td>56237</td>
<td>0</td>
<td>Andrew Keats</td>
<td>Study Report for ENS-1001 To determine the formulation stability profile of the APITHOR Hive Beetle Harbourage, by monitoring changes in chemical and physical properties following accelerated storage conditions of 54 plus or minus 2 Degrees C for a period of 14 days</td>
<td>11 May 11</td>
<td>Chemistry and Manufacture</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
<td>56238</td>
<td>0</td>
<td>Anon</td>
<td>APITHOR PRODUCTION - THAILAND</td>
<td>14 January 2011</td>
<td>Chemistry and Manufacture</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
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<td>0</td>
<td>Andrew Keats</td>
<td>Certificate of Analysis - ASA-06-185B</td>
<td>6/11/06</td>
<td>Residues</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
<td>56240</td>
<td>0</td>
<td>Garry Levot</td>
<td>FIELD TRIAL TO MEASURE FIPROLE RESIDUES IN HONEY AND WAX FROM APITHOR TREATED BEE HIVES.</td>
<td>6/11/06</td>
<td>Residues</td>
<td>2018-12-09</td>
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</tr>
<tr>
<td>54227</td>
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<td>Garry Levot</td>
<td>EFFECTIVENESS OF THE INSECTICIDAL SMALL HIVE BEETLE REFUGE TRAP APITHOR IN REDUCING ADULT BEETLE NUMBERS IN BEE HIVES.</td>
<td>July 11</td>
<td>Efficacy and Safety</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
<td>56243</td>
<td>0</td>
<td>Garry Levot</td>
<td>FIELD TRIAL TO MEASURE THE SAFETY OF APITHOR TO BEES</td>
<td>July 11</td>
<td>Efficacy and Safety</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
<td>56246</td>
<td>0</td>
<td>Andrew Keats</td>
<td>Determination of residues of fipronil in honey and wax specimens submitted for analysis</td>
<td>4/9/11</td>
<td>Residues</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
</tbody>
</table>
### Validation and description of laboratory analytical method for: ALM-FIP-10-01

**Determination of Active Ingredient Fipronil in Cardboard Based Formulation**

<table>
<thead>
<tr>
<th>Code</th>
<th>Name</th>
<th>Description</th>
<th>Date</th>
<th>Department</th>
<th>Date of Approval</th>
<th>Registrant/ Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>54227</td>
<td>Anon.</td>
<td>Validation and description of laboratory analytical method for: ALM-FIP-10-01 Determination of Active Ingredient Fipronil in Cardboard Based Formulation</td>
<td>August 2012</td>
<td>Chemistry and Manufacture</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144 AUSTRALIA</td>
</tr>
<tr>
<td>54227</td>
<td>Gary Levot</td>
<td>APITHOR SAFETY AND RESIDUE TRIALS 2012</td>
<td>14 May 2013</td>
<td>Efficacy and Safety</td>
<td>2018-12-09</td>
<td>Registrant/ UNIT 3, THE JUNCTION ESTATE 4-6 JUNCTION STREET AUBURN NSW 2144</td>
</tr>
</tbody>
</table>

### Withholding period details

**WITHHOLDING PERIOD NOT REQUIRED WHEN USED AS DIRECTED**

### Host/pest details

<table>
<thead>
<tr>
<th>Host</th>
<th>Pest</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEEHIVE</td>
<td>[BEETLE - SMALL HIVE]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Host alias</th>
<th>Pest alias</th>
</tr>
</thead>
<tbody>
<tr>
<td>No host alias</td>
<td>No pest alias</td>
</tr>
</tbody>
</table>
16 Appendix 5: Chemical permit labels and MSDS

16.1 Bayvarol label

Bayvarol® Strips

Parasiticide for the diagnosis and therapy of flumethrin sensitive Varroa on bees.

Directions for use
By law, the label directions must be followed. Do not open the strips until immediately prior to use. Once opened, the strips should be used and discarded within 6 – 8 weeks.

Bayvarol strips are suspended into the spaces between the combs in the central brood rearing area (i.e. not in the honey supers) in such a way that they can be occupied by bees on both sides. Bend both tabs outwards in the same direction at the marked fold lines and hook over the top edge of the wooden frame. (See Figure 1)

For large colonies occupying several brood chambers two strips can be joined together end to end which enables their insertion into and removal from the bee spaces without having to separate the brood chambers. (See Figure 2)

Figure 1

Figure 2

Dosage
Normally developed colonies receive four (4) strips per brood chamber. Nuclei and young colonies and newly collected swarms use two (2) strips per chamber (half dose).
Large colonies occupying several brood chambers use four strips per chamber, which are distributed over the central bee spaces in each brood chamber. Avoid the strips coming into contact with honey to be harvested for human consumption.

When to use
Best efficacy is to be expected when Bayvarol is used in the late summer after the honey harvest. The strips should not be used during peak honey flow periods. However, Bayvarol can be used at any time of year for diagnosis or in severe infestations where there is a threat to the survival of the colony. If emergency treatment for an infestation is necessary while honey supers are present comb honey should not be sold. It is important the strips are not re-used.

1. Diagnosis
Bayvarol Strips are inserted into the colony for 24 hours. Before inserting the strips cover the floor tray with a clean sheet of paper. This is then checked for the presence of dead Varroa mites 24 hours later.
2. Treatment
Bayvarol Strips should be left in the colonies for six to eight weeks and then removed.

Resistance Management
Intensive use of Bayvarol could result in the development of resistant strains of mites. To minimise this risk use Bayvarol strictly in accordance with the label directions. It is a requirement to alternate the use of Bayvarol with products from other chemical groups.

Flumethrin belongs to the synthetic pyrethroid group of chemicals.

Precautions
Wash hands after handling the strips, before meals and after work.

Environmental Protection
Harmful to aquatic life with long lasting effects. Avoid contamination of any water supply with product. Avoid release to the environment.

Storage
Store below 25°C in a cool dry place away from food.

Disposal
Dispose of used strips safely by wrapping in paper and placing them in domestic refuse.

Bayer New Zealand Limited
Argus Place, Hillocks, North Shore City 0627
09 443 3093

Approved under the Animal Products (Ancillary and Transitional Provisions) Act 1999
Registered pursuant to the ACVM Act 1997, No. P5683
See www.nchspa.govt.nz/acvm/ for registration conditions.

BAYVAROL is a trademark of BAYER AG, Leverkusen, Germany.
16.2 Apistan label

![Apistan label image]

**SPECIMEN LABEL**

- **ACTIVE INGREDIENT:**
  - Tetrafluorurate (CAS #102851-06-9) 10.25%
  - OTHER INGREDIENTS: 89.75%

- **Total:** 100.00%

- **EPA Reg. No:** 27244-06

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION**

See additional precautions/directions

**PRECAUTIONARY STATEMENTS – HAZARDS TO HUMANS AND DOMESTIC ANIMALS – CAUTION**

- Harmful if swallowed. Harmful if absorbed through skin. Causes moderate eye irritation. Avoid contact with eyes, skin, or clothing. If workers contact treated material or surfaces: pesticide residues that get on their skin may cause itching and/or irritation that can be severe and they should avoid contact with treated strips or surfaces, and they should wash the affected skin immediately. If irritation begins to occur.

**FIRST AID:** Call a poison control center or doctor immediately for treatment advice.

**IF SWALLOWED**

- Call a poison control center or doctor immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by the poison control center or doctor.
- Do not give anything by mouth to an unconscious person.

**IF IN EYES**

- Hold eye open and rinse slowly and gently with water for 15-20 minutes.
- Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.

**Personal Protective Equipment (PPE):** Some materials that are chemical-resistant to this product are those made of any waterproof material. If you want more options, follow the instructions for category A on a chemical-resistance category selection chart.

**All handlers must wear:** Long-sleeved shirt and long pants, shoes and socks, chemical-resistant gloves

Follow manufacturer’s instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**USER SAFETY RECOMMENDATIONS:** User should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.

User should remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

User should remove PPE immediately after handling this product. Wash the outside of gloves before
removing. As soon as possible, wash thoroughly and change into clean clothing.

**ENVIRONMENTAL HAZARDS**

This product is toxic to honey bees if bees are exposed to direct application. However, dried residues of this product are non-toxic to honey bees. Treat during non-foraging periods to minimize adverse effects.

**DIRECTIONS FOR USE**

It is a violation of Federal Law to use this product in a manner inconsistent with its label.

Just before application, remove the required number of APISTAN® Strips from the pouch. To separate strips, hold firmly at corner, near tab, and pull along scored line, from top to bottom. Unused strips should remain in original package.

Do not place strips in direct contact with combs containing honey intended for human consumption. After treatment, do not use beeswax for human consumption (including honeycomb, chunk honey, and wax for confectionary purposes).

Use one strip for each 5 combs of bees or less in each brood chamber (Langstroth deep frames or equivalent in other sizes). Hang the strips within two combs of the edge of the bee cluster. APISTAN® Strips must be in contact with brood nest bees at all times. If two deep supers are used for the brood nest, hang APISTAN® Strips in alternate corners of the cluster, in the top and bottom super. For best chemical distribution, use APISTAN® Strips when daytime high temperatures are at least 50° F.

**FOR CONTROL**

Remove honey supers before application of APISTAN® Strips and do not replace until the end of the control period. For adequate control within a brood yard, treat all infested colonies within that yard.

Effective control may be achieved by treating hives in the spring before the first honey flow and in the fall after the last honey flow. Do not remove strips from hive for at least 42 days (6 weeks). Do not leave strips in hive for more than 56 days (8 weeks). Honey supers may be replaced after strips are removed.

**FOR DETECTION**

Place white sticky paper below the frames (sticky side up). Place APISTAN® Strips as per above instructions. At various intervals (up to 7 days), check for Varroa on withdrawn white, sticky paper from below the frames.

---

**STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

**PESTICIDE STORAGE**

Store in a cool, dry place. Keep strips in original unopened package until ready to use. The storage area must be dry, well-lit, and well-ventilated. Do not store in direct sunlight. Do not store unused strips in anything but original package. Do not store unused strips near pesticides or other chemical substances that could contaminate the strips and result in bee toxicity. Keep pesticide storage areas clean.

**PESTICIDE DISPOSAL**

To avoid wastes, use all material in this container by application according to label directions. If wastes cannot be avoided, offer remaining product to a waste disposal facility or pesticide disposal program (often such programs are run by state or local governments or by industry).

**CONTAINER HANDLING**

Nonrefillable container. Do not reuse or refill this container. Do not reuse strips. Do not for recycling if available or dispose of empty carton in a sanitary landfill or by incineration or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

To the extent consistent with applicable law, Seller makes no warranty, expressed or implied, concerning the use of this product other than indicated on the label. To the extent consistent with applicable law, Buyer shall assume risks of use and handling of this material when such use and handling are contrary to label instructions. Always read the label before using the product.

For information or in case of emergency, call 1-800-248-7763.

www.centralapiary.com

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Wellmark International
1501 East Woodfield Road 200W
Schaumburg, Illinois 60173

ZOECON.

Apistan, the Apistan logo and the Zoecon logo are registered trademarks of Wellmark International.

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October, 2010
Schaumburg, Ill.
16.3 Apithor label

![Apithor Label Image]
SECTION 1 – Identification, Contacts, Hazardous Nature

<table>
<thead>
<tr>
<th>Bayer New Zealand Ltd</th>
<th>Telephone No:</th>
<th>09 443 3093</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>National Poisons and Hazchem Information Centre:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0800 POISON</td>
<td>(0800 764 766)</td>
</tr>
<tr>
<td></td>
<td>Emergency No. (24 hours) : 0800 734 607</td>
<td></td>
</tr>
<tr>
<td>Product Name</td>
<td>Bayvarol Strips</td>
<td></td>
</tr>
<tr>
<td>Product Use</td>
<td>For the diagnosis and control of Varroa mites on honey bees.</td>
<td></td>
</tr>
<tr>
<td>Other Names</td>
<td>0.54 g/kg flumethrin in the form of an impregnated polymer strip</td>
<td></td>
</tr>
<tr>
<td>Creation Date</td>
<td>22nd October 2004</td>
<td></td>
</tr>
<tr>
<td>Revision Date</td>
<td>22nd October 2004</td>
<td></td>
</tr>
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</table>

SECTION 2 – Hazards Identification

<table>
<thead>
<tr>
<th>HSNO Classification</th>
<th>9.1D</th>
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<tbody>
<tr>
<td></td>
<td>Harmful to aquatic life.</td>
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</tbody>
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SECTION 3 – Composition Information on Ingredients

<table>
<thead>
<tr>
<th>flumethrin</th>
<th>weight %: &lt; 0.25 CAS No.: 69770-45-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-phrases: 25-50/53</td>
</tr>
<tr>
<td>acetone</td>
<td>weight % &lt; 15 CAS No.: 67-64-1</td>
</tr>
<tr>
<td></td>
<td>R-phrases: 11-36-66-67</td>
</tr>
</tbody>
</table>
### SECTION 4 – First Aid measures

| Skin Contact | If poisoning occurs contact a doctor or Poisons Information Centre 0800 POISON  
| Skin Contact | Remove soiled or soaked clothing immediately  
| Eye Contact | After contact with skin, wash immediately with plenty of water and soap.  
| Eye Contact | Contamination of the eyes must be treated by thorough irrigation with water, with the eyelids held open. A doctor (or eye specialist) should be consulted immediately.  
| Ingestion | If swallowed, seek medical advice immediately and show safety data sheet.  

### SECTION 5 – Fire Fighting Measures

| Extinguishing media | Sprayed water, jet foam, extinguishing powder, CO₂, sand  
| Combustion gases | In case of fire carbon monoxide, hydrogen chloride, hydrogen and nitrogen oxides may develop. Firemen have to wear self-contained breathing apparatus.  
| Further information | In case of fire care must be taken to collect the fire water.  

### SECTION 6 – Accidental Release Procedures

| Accidental Release | Use any personal protective equipment listed in Chapter 8  
| Accidental Release | If larger quantities are released prevent from entering waterways, sewage systems, surface water and or ground water.  
| Accidental Release | Take up mechanically, fill into labelled closable containers. Avoid formation of dust.  
| Accidental Release | Do not eat, drink or smoke during clean up operation.  
| Accidental Release | To clean the floors and all objects contaminated by this material use water and detergents.  

### SECTION 7 – Handling and Storage

| Safe handling                      | During normal use the packaging ensures safe handling. Follow instructions and product label. |
| Fire and Explosion Prevention      | Avoid creation of electrostatic charges when handling. Dust can combine with air to form an explosive mixture. Keep away from sources of ignition. |
| Storage                            | Keep out of reach of children |
|                                   | Store away from food, drink or animal feeding stuffs. |
|                                   | To maintain product quality store below 40°C and keep dry. |
|                                   | Protect from temperatures below 0°C. |
|                                   | Store cool and dry in original tightly closed containers. |
|                                   | Store away from incompatible materials (section 10) |
|                                   | Where quantities > 10,000 kgs are stored HSNO compliant emergency response plans and signage are required. |

### SECTION 8 – Exposure Control And Personal Protection

| Exposure Control                   | When handling the product ensure adequate ventilation and provide air extraction. |
| Acetone                            | MAK-value: 500ppm (1200 mg/m³) |
| Personal protective clothing and equipment | Under normal conditions of use no personal protective equipment is required. |
| Respiratory Equipment              | Under other conditions wear garments usually required within the Pharmaceutical Industry. |
| Eye Protection                     | Half mask with filter type P3 |
| Hand protection                    | Goggles |
|                                   | Protective gloves for chemicals made of Baypren, nitrile rubber or PVC wear |
|                                   | After contamination of gloves with product change them. The gloves should not be reused. |
Cleanliness Guidelines (GMP) for manufacturing of drugs must be observed!

**SECTION 9 – Physical and Chemical Properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form</td>
<td>solid, plastic panel (polyethylene)</td>
</tr>
<tr>
<td>Colour</td>
<td>turbid, white</td>
</tr>
<tr>
<td>Odour</td>
<td>weak odour</td>
</tr>
<tr>
<td>Melting point</td>
<td>approx. 120 °C</td>
</tr>
<tr>
<td>Density</td>
<td>No statements available</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>No statements available</td>
</tr>
<tr>
<td>Solubility with water</td>
<td>insoluble</td>
</tr>
<tr>
<td>pH value</td>
<td>No statements available</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>Log P octanol / water = flumethrin: 6.2</td>
</tr>
<tr>
<td>Flash point</td>
<td>No statements available</td>
</tr>
<tr>
<td>Ignition temperature</td>
<td>No statements available</td>
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</table>

**SECTION 10 – Stability and Reactivity**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Stability</td>
<td>Product is stable. No hazardous reactions.</td>
</tr>
<tr>
<td>Incompatible Materials</td>
<td>None</td>
</tr>
<tr>
<td>Conditions to avoid</td>
<td>Avoid heat and moisture</td>
</tr>
<tr>
<td>Hazardous decomposition products</td>
<td>Formation of hydrogen chloride, hydrogen cyanide, carbon monoxide and nitrogen oxides possible during thermal decomposition.</td>
</tr>
</tbody>
</table>

**SECTION 11 – Toxicological Information**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute toxicity flumethrin</td>
<td></td>
</tr>
<tr>
<td>LD₅₀ oral, rat</td>
<td>&gt;= 100 mg/kg (Bayer)</td>
</tr>
<tr>
<td>LD₅₀ dermal, rat</td>
<td>&gt; 2000 mg/kg (Bayer)</td>
</tr>
<tr>
<td>Skin and mucous membrane compatibility</td>
<td></td>
</tr>
<tr>
<td>flumethrin</td>
<td>Irritant effects on the eyes</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Irritant effects on the skin</td>
</tr>
<tr>
<td></td>
<td>Skin sensitisation</td>
</tr>
</tbody>
</table>

### SECTION 12 – Ecotoxicity Information

<table>
<thead>
<tr>
<th>Fish toxicity flumethrin</th>
<th>Do not allow to enter surface waters or groundwater.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC₅₀: 0.17 mg/l (96 h); Rainbow trout (oncorhynchus mykiss)</td>
<td></td>
</tr>
<tr>
<td>Toxicity for Daphnia flumethrin</td>
<td>EC₅₀: 0.027 mg/l (48 h); Water flea (Daphnia magna) (Bayer)</td>
</tr>
<tr>
<td>Toxicity for algae flumethrin</td>
<td>Growth rate:</td>
</tr>
<tr>
<td>IC₅₀: 0.59 mg/l (72 h); Green algae (Desmodesmus subspicatus) (Bayer)</td>
<td></td>
</tr>
</tbody>
</table>

### SECTION 13 – Disposal information

<table>
<thead>
<tr>
<th>After Intended Use</th>
<th>Dispose of used strips in an appropriate landfill facility or through domestic garbage collection.</th>
</tr>
</thead>
<tbody>
<tr>
<td>After spill or accident</td>
<td>Dispose of sealed containers at an approved local waste disposal site.</td>
</tr>
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</table>

### SECTION 14– Transport information

<table>
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<tr>
<th>UN Number</th>
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<tr>
<td>UN Proper shipping Name</td>
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</tr>
<tr>
<td>Class</td>
<td>Not classified</td>
</tr>
<tr>
<td>Packaging Group</td>
<td>Not classified</td>
</tr>
<tr>
<td>Hazchem Code</td>
<td>Not classified</td>
</tr>
<tr>
<td>Declaration for transport</td>
<td>Not required</td>
</tr>
<tr>
<td>Other information</td>
<td>Not dangerous for transport. Slightly smelling. Keep dry. Keep separated from foodstuffs.</td>
</tr>
</tbody>
</table>
SECTION 15 – Regulatory Information

<table>
<thead>
<tr>
<th>ACVM Registration</th>
<th>No. P5683</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSNO Approval Code</td>
<td>HSR000756</td>
</tr>
</tbody>
</table>

SECTION 16 – Other Information

Text of all R phrases referred to in sections 2 and 3:

- R11: Highly flammable.
- R 23/25: Also toxic by inhalation and if swallowed
- R 36: Irritating to eyes.
- R 37: Irritating to respiratory system.
- R 50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
- R 66: Repeated exposure may cause skin dryness or cracking.
- R 67: Vapours may cause drowsiness and dizziness.

The data given here is based on current knowledge and experience. The purpose of this Safety Data Sheet is to describe the products in terms of their safety requirements. The above details do not imply any guarantee concerning composition, properties or performance.
### 16.5 Apistan MSDS

| Date Issued: | September, 2010 |
| Supersedes: | November, 2004 |

**MATERIAL SAFETY DATA SHEET**

**APISTAN® ANTI-VARROA MITE STRIP**

**Manufacturer:** Wellmark International  
**Address:** 1501 E. Woodfield Rd., Suite 200 W, Schaumburg, IL 60173  
**Emergency Phone:** 1-800-248-7763  
**Transportation Emergency Phone:** CHEMTREC: 1-800-424-9300

#### 1. CHEMICAL PRODUCT INFORMATION

| Product Name: | Apistan® Anti-Varroa Mite Strip |
| Chemical Name/Synonym: | tau-Fluvalinate: (RS)-α-cyano-3-phenoxybenzl N-(2-chloro-α,α,α-trifluoro-p-tolyl)-D-valinate |
| Chemical Family: | Synthetic pyrethroid |
| Formula: | C26 H22 Cl F3 N2 O3 |
| EPA Registration No.: | 2724-406 |
| RF Number: | 318G |
| Inventory Control Number | 300507997 |

#### 2. COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Component (chemical, common name) Number</th>
<th>CAS</th>
<th>Weight Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>tau-Fluvalinate: (RS)-α-cyano-3-phenoxybenzl N-(2-chloro-α,α,α-trifluoro-p-tolyl)-D-valinate</td>
<td>102851-06-9</td>
<td>10.25 % Not established</td>
</tr>
<tr>
<td>Inert Ingredients (non-hazardous/trade secret)</td>
<td></td>
<td>89.75 %</td>
</tr>
</tbody>
</table>
3. HAZARD INFORMATION

PRECAUTIONARY STATEMENT
KEEP OUT OF REACH OF CHILDREN.
HAZARDS TO HUMANS AND DOMESTIC ANIMALS- CAUTION:
Harmful if swallowed. Harmful if absorbed through skin. Causes moderate eye irritation. Avoid contact with eyes, skin, or clothing. If workers contact treated material or surfaces: pesticide residues that get on their skin may cause itching and/or irritation that can be severe and they should avoid contact with treated strips or surfaces, and they should wash the affected skin immediately if irritation begins to occur.

SIGNS AND SYMPTOMS OF OVEREXPOSURE
Clinical symptoms may include salivation, depression, labored breathing, diarrhea. In certain individuals, a temporary sensory effect (itching, tingling, and numbness) may occur which usually subsides without medical treatment.

PRIMARY ROUTE OF ENTRY  
Dermal/Eye: Yes
Oral: No
Inhalation: No

ACUTE TOXICITY
Oral: Harmful if swallowed
Dermal: Harmful if absorbed through the skin
Inhalation: No specific hazard identified

OTHER TOXICOLOGICAL INFORMATION
Skin Irritation: May cause severe skin irritation
Eye Irritation: Causes moderate eye irritation
Sensitizer: Not a skin sensitizer

4. FIRST AID MEASURES
If in eyes:  
• Hold eye open and rinse slowly and gently with water for 15-20 minutes.
• Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eyes.

If on Skin:  
• Take off contaminated clothing.
• Rinse skin immediately with plenty of water for 15-20 minutes.
• Call a poison control center or doctor for treatment advice.

If swallowed:  
• Call a poison control center or doctor immediately for treatment advice.
• Have person sip a glass of water if able to swallow.
• Do not induce vomiting unless told to do so by the poison control center or doctor.
• Do not give anything by mouth to an unconscious person.

5. FIRE FIGHTING MEASURES
NFPA Rating: Health: 1 Fire: 0 Reactivity: 0
Flammability Class: Combustible solid
Flash Point: Does not flash
Explosive Limits (% of Volume): Not established
6. ACCIDENTAL RELEASE MEASURES

Steps to be taken: Because of product form and packaging, possibilities of a spill are remote. However, should one occur, wear proper protective equipment (See Special Protective Equipment) and sweep up spill. Place in a container for disposal.

Absorbents: Due to product form, no absorbents should be necessary. If in liquid form, use clay granules, sawdust, dirt or equivalent.

Incompatibles: Strong acids or bases

7. HANDLING AND STORAGE

Handling: Avoid contact with eyes, skin or clothing. Wear appropriate chemical resistant gloves when handling the strips. Wash hands, face and arms thoroughly with soap and water after handling product. Container Handling: Non-refillable container. Do not reuse or refill this container. Do not reuse strips.

Storage: Store in a cool, dry place. Keep in original unopened package until ready to use. Storage area must be dry, well-lit, and well-ventilated. Do not store in direct sunlight. Do not store unused strips in anything but original package. Do not store unused strips near pesticides or other chemicals that could contaminate the strips and result in bee toxicity. Keep storage areas clean.

Exposure Limits: Not established

Ventilation: Provide mechanical ventilation when handling

Personal Protective Equipment: Wear appropriate chemical resistant gloves. All handlers must wear: Long-sleeved shirt and long pants, Shoes and socks, and chemical-resistant gloves. Keep and wash PPE separately from other laundry.

8. PHYSICAL AND CHEMICAL PROPERTIES

Appearance and Odor: Light golden colored, clear plastic strip, very low odor.

Boiling Point: N/A

Melting Point: N/A

Vapor Pressure (mm Hg): Not determined

Vapor Density (Air = 1): Not determined

Specific Gravity: N/A

Bulk Density: about 1.14 g/cc

Solubility: None

Evaporation Rate: N/A

pH: N/A

9. STABILITY AND REACTIVITY

Stability: Stable

Reactivity: Not reactive
**Incompatibility w/Other Materials:**
- Strong oxidizing agents

**Decomposition Products:**
- Hydrogen cyanide, hydrogen fluoride,
- hydrogen chloride, carbon monoxide, carbon dioxide

**Hazardous Polymerization:**
- Will not occur

### 10. TOXICOLOGICAL INFORMATION

#### ACUTE TOXICITY [Based on tau-fluvalinate]
- Acute oral toxicity: LD50 = 1402 mg/kg
- Acute dermal toxicity: LD50 >2000 mg/kg
- Acute inhalation: No data – tau-fluvalinate is viscous liquid with vapor pressure >1 X 10^{-7} torr at 25°C Skin irritation: Mild or slight irritation
- Eye irritation: Moderate eye irritant
- Not a dermal sensitizer

#### CHRONIC TOXICITY [Based on tau-fluvalinate]
Rats received tau-fluvalinate via gavage. No oncogenic potential was shown. The NOEL was 1 mg/kg/day.

Mice were fed diets containing tau-fluvalinate. With the exception of skin lesions, no compound-related toxicity was observed. No oncogenic potential was shown. The systemic NOEL was considered to be 20 mg/kg/day.

Dogs were treated with racemic tau-fluvalinate daily for six months. Vomiting and diarrhea occurred at 50 mg/kg/day and skin lesions at the 3 highest levels. The NOEL was determined to be 2 mg/kg/day.

#### DEVELOPMENTAL/REPRODUCTIVE TOXICITY [Based on tau-fluvalinate]
Rats were administered racemic tau-fluvalinate during presumed gestation. The developmental NOEL was 10 mg/kg/day. Skin lesions were observed at 100 ppm and above. Treatment-related mortality occurred at 500 and 1000 ppm. In the first generation, pup growth was inhibited at levels of 250 ppm and above during lactation. The NOEL was 20 ppm.

Rabbits were administered tau-fluvalinate during presumed gestation. Signs of maternal toxicity were anorexia, depression, and decreased body weights. The NOEL was 25 mg/kg/day.

#### MUTAGENICITY [Based on tau-fluvalinate]
The weight of evidence suggests tau-fluvalinate is not a mutagen.

### 11. ECOLOGICAL INFORMATION

#### ENVIRONMENTAL FATE [Active Ingredients Only]
- **Hydrolysis:** Main mechanism of dissipation at basic pH
- **Photolysis:** Degraded rapidly in sunlight and artificial light
- **Soil half life:** 6-15 days depending on soil type and oxygen
- **Water solubility:** 0.002 mg/L at 25°C

#### ECOTOXICITY [Active Ingredients Only]
- **Acute Toxicity:**
  - fish LC50: rainbow trout 0.91 ug/L (96 hour study); bluegill 1.2 ug/L (96 hour study); sheephead minnow 1.5 ug/L (96 hour study)
  - aquatic invertebrates LC50: Daphnia magna 0.94 ug/L (48 hour study); Mysis shrimp 0.1179 ug/L (96 hour study)

### 12. DISPOSAL CONSIDERATIONS

Wastes resulting from use of this product should be disposed of in accordance with all federal, state and local requirements. For additional regulatory information, see section 14 of this document.
13. TRANSPORT INFORMATION

**DOT49CFR Description:** Not regulated as hazardous by D.O.T.

**Freight Classification:** Insecticide, NOI, other than poison in boxes. NMFC 1055050

14. REGULATORY INFORMATION

**CERCLA (Superfund):** Not regulated

**RCRA:** Not regulated

**SARA 311/312 HAZARD CATEGORIES**

**Immediate Health:** Yes (irritant)

**Delayed Health:** No

**Fire:** No

**Sudden Pressure:** No

**Reactivity:** No

The information presented herein, while not guaranteed, was prepared by technically knowledgeable personnel and to the best of our knowledge is true and accurate. It is not intended to be all inclusive and the manner and conditions of use and handling may involve other or additional considerations.
## 16.6 Apithor MSDS

### Material Safety Data Sheet

APITHOR* Hive Beetle Harbourage

### Section 1 - IDENTIFICATION OF CHEMICAL PRODUCT AND COMPANY

This product is NOT classified as Hazardous according to the criteria of NOHSC Australia. Not a Dangerous Good according to the Australian Dangerous Goods (ADG) Code.

Ensystex Australasia Pty Ltd  
Unit 3 The Junction Estate  
4 – 6 Junction Street  
AUBURN NSW 2144  
Tel: 13 35 36 (24 hours)

**Substance:** Ready-to-use Harbourage for the control of the small hive beetle in bee hives.  
**Trade Name:** APITHOR Hive Beetle Harbourage.  
**Product Use:** Insecticidal Harbourage for use as described on the product label.  
**Creation Date:** August 2010  
**Revision Date:**

### Section 2 - HAZARDS IDENTIFICATION

**Statement of Hazardous Nature**  
This product is NOT classified as hazardous according to the criteria of NOHSC Australia. Not a Dangerous Good according to the Australian Dangerous Goods (ADG) Code.

**Safety Phrases:** None.  
**SUSDP Classification:** UNSCHEDULED (Standard for the Uniform Scheduling of Drugs and Poisons).  
**ADG Classification:** None allocated. Not a Dangerous Good.  
**UN Number:** None allocated.

### Emergency Overview

**Physical Description & colour:** Black plastic harbourage with insecticide impregnated cardboard insert.  
**Odour:** None.  
**Major Health Hazards:** None.

### Potential Health Effects

**Inhalation:** Not applicable.  
**Skin Contact:** Not a skin irritant.
**Eye Contact:** Unlikely to irritate eyes.

**Ingestion:** Low acute oral toxicity.

**Carcinogen Status:**
- **NOHSC:** No significant ingredient is classified as carcinogenic by NOHSC.
- **NTP:** No significant ingredient is classified as carcinogenic by NTP.
- **IARC:** No significant ingredient is classified as carcinogenic by IARC.

### Section 3 - COMPOSITION/INFORMATION ON INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CAS No</th>
<th>Conc,%</th>
<th>TWA (mg/m³)</th>
<th>STEL (mg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fipronil</td>
<td>120068-37-3</td>
<td>0.048%</td>
<td>not set</td>
<td>not set</td>
</tr>
<tr>
<td>Cardboard</td>
<td>N/A</td>
<td></td>
<td>not set</td>
<td>not set</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>9003-07-0</td>
<td></td>
<td>not set</td>
<td>not set</td>
</tr>
</tbody>
</table>

This is a commercial product whose exact ratio of components may vary slightly.

The TWA exposure value is the average airborne concentration of a particular substance when calculated over a normal 8 hour working day for a 5 day working week. The STEL (Short Term Exposure Limit) is an exposure value that should not be exceeded for more than 15 minutes and should not be repeated for more than 4 times per day. There should be at least 60 minutes between successive exposures at the STEL. The term "peak" is used when the TWA limit, because of the rapid action of the substance, should never be exceeded, even briefly.

### Section 4 - FIRST AID MEASURES

**General Information:**
You should call The Poisons Information Centre if you feel that you may have been poisoned or irritated by this product. The number is 13 11 26 from anywhere in Australia (0800 764 766 in New Zealand) and is available at all times. Have this MSDS with you when you call.

**Inhalation:** First aid is not generally required. If in doubt, contact a Poisons Information Centre or a doctor.

**Skin Contact:** Irritation is not expected. If any unusual symptoms become evident, or if in doubt, wash skin with soap and water. If symptoms persist, contact a Poisons Information Centre or a doctor.

**Eye Contact:** Unlikely. If irritation does occur, flush eye(s) with lukewarm, gently flowing water for 5 minutes. Obtain medical advice if irritation becomes painful or lasts more than a few minutes.

**Ingestion:** Unlikely. If product gets in mouth, wash mouth with water and spit out. Contact a Poisons Information Centre or a doctor.

**Advice to Doctor:** Fipronil is a reversible gamma-aminobutyric (GABA) receptor inhibitor. During intoxication it will induce neurological stimulation with possible convulsions. Treat symptoms. No specific antidote known. Phenobarbital, and to a lesser
extent, benzodiazepines, have been shown experimentally to be effective in preventing convulsions induced by fipronil. Due to slow absorption of fipronil through the gut, symptoms of intoxication may be delayed several hours to one day. Absorption may be decreased by the use of gastric lavage, saline purgative and activated charcoal (possible enterohepatic recirculation). Continue monitoring due to slow elimination of the compound.

Section 5 - FIRE FIGHTING MEASURES

**Fire and Explosion Hazards:** There is no risk of an explosion from this product under normal circumstances if it is involved in a fire. Fire decomposition products from this product may be toxic if inhaled. Take appropriate protective measures.

**Extinguishing Media:** Preferred extinguishing media are carbon dioxide, dry chemical, foam, water fog.

**Fire Fighting:** If a significant quantity of this product is involved in a fire, call the fire brigade.

**Flash point:** Not flammable.

**Upper Flammability Limit:** Does not burn.

**Lower Flammability Limit:** Does not burn.

**Autoignition temperature:** Does not burn.

**Flammability Class:** Does not burn.

Section 6 - ACCIDENTAL RELEASE MEASURES

**Accidental release:** Not possible.

Section 7 - HANDLING AND STORAGE

**Handling:** The measures detailed below under "Storage" should be followed during handling in order to minimise risks to persons using the product in the workplace. Also, avoid contact or contamination of product with incompatible materials listed in Section 10.

**Storage:** KEEP OUT OF THE REACH OF CHILDREN. Store in the closed, original container in a dry, cool, well-ventilated area out of direct sunlight. Dispose of used product by wrapping in paper, placing in a plastic bag and place in a garbage bin. Check packaging - there may be further storage instructions on the label.

Section 8 - EXPOSURE CONTROLS AND PERSONAL PROTECTION

No special equipment is usually needed. The following instructions are for bulk handling or where regular exposure in an occupational setting occurs.

**Ventilation:** No special ventilation requirements are necessary for this product.

**Eye Protection:** Eye protection is not necessary when this product is being used.
Skin Protection: The information at hand indicates that this product is not harmful and that no special skin protection is necessary.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

Physical Description & colour: Sealed black plastic harbourage with insecticide impregnated cardboard insert.

Odour: None.

Boiling Point: N/A.

Freezing/Melting Point: N/A.

Vapour Pressure: Expected to be negligible at 100°C.

Boiling Point: N/A.

Freezing/Melting Point: N/A.

Vapour Pressure: Negligible at normal ambient temperatures.

Water Solubility: Largely insoluble.

pH: No data. Expected to be neutral.

Volatility: Negligible at normal ambient temperatures.

Section 10 - STABILITY AND REACTIVITY

Reactivity: This product is unlikely to react or decompose under normal storage conditions. However, if you have any doubts, contact the supplier for advice on shelf life properties.

Conditions to Avoid: Containers should be kept dry. Store in the closed original container in a dry, cool, well-ventilated area out of direct sunlight.

Incompatibilities: Strong acids, strong bases, strong oxidising agents.

Fire Decomposition: No specific data. The following might be expected: Carbon dioxide, and if combustion is incomplete, carbon monoxide and smoke.

Section 11 - TOXICOLOGICAL INFORMATION

Toxicity: Acute dermal: low, LD50 >2000 mg/kg bw (rabbit),

Acute inhalation: LC50 (4 hr) >1.7 mg/L (Rat)

Acute inhalation: LC50 (1 hr, calculated) >6.8 mg/L (Rat)

Skin Sensitisation: Non-sensitizing (Guinea pig)

Section 12 - ECOLOGICAL INFORMATION

Non-toxic to bees or honey in presentation provided. Non-toxic to earthworms. Toxic to fish and aquatic organisms. Do NOT apply to areas where surface water is present. Do NOT contaminate streams, rivers or waterways with the product or used containers.
Section 13 - DISPOSAL CONSIDERATIONS

Disposal: Dispose of used product by wrapping in paper, placing in a plastic bag and place in a garbage bin. Further instructions concerning the disposal of this product and its container may be given on the product label. These should be carefully followed.

Section 14 - TRANSPORT INFORMATION

ADG Code: This product is not a Dangerous Good. No special transport conditions necessary.

Section 15 - REGULATORY INFORMATION

AICS: All of the significant ingredients in this formulation are to be found in the public AICS Database.

Section 16 - OTHER INFORMATION

This MSDS contains only safety-related information. For other data see product literature.

Acronyms:

- **ADG Code**: Australian Code for the Transport of Dangerous Goods by Road and Rail
- **AICS**: Australian Inventory of Chemical Substances
- **CAS number**: Chemical Abstracts Service Registry Number
- **Hazchem Number**: Emergency action code of numbers and letters that provide information to emergency services especially firefighters
- **IARC**: International Agency for Research on Cancer
- **NOHSC**: National Occupational Health and Safety Commission
- **NOS**: Not otherwise specified
- **NTP**: National Toxicology Program (USA)
- **R-Phrase**: Risk Phrase
- **SUSDP**: Standard for the Uniform Scheduling of Drugs & Poisons
- **UN Number**: United Nations Number

This MSDS summarises our best knowledge of the health and safety hazard information of the product and how to safely handle and use the product in the workplace. Each user must review this MSDS in the context of how the product will be handled and used in the workplace.

If clarification or further information is needed to ensure that an appropriate risk assessment can be made, the user should contact Ensystex so we can attempt to obtain additional information from our suppliers. Our responsibility for products sold is subject to our standard terms and conditions, a copy of which is sent to our customers and is also available on request.

Please read all labels carefully before using product.
17 References


Plant Health Australia (2012). *Threat Specific Contingency Plan - Tracheal mite Acarapis woodi*. Plant Health Australia, Canberra, ACT.


