# **Bee Informed Partnership**Pesticide Sampling Protocol



# **Sampling for Pesticide Residue**

The most common pesticide load testing is conducted either bee bread or wax. Other types of testing can be performed on bees, queens and honey etc. Note: this analysis may not be conclusive; these results may not give you a definitive answer to the question, "Why are some of my bees dying?" Some pesticides break down quickly so they will not be detected, others have unknown effects on bees at low doses or in combination with other pesticides. This analysis is meant to be informative, helping rule out some potential causes of loss.

\*This sample can be a combined (composite) sample from the healthy or declining colonies you sampled to gather your live bee and alcohol samples.

# **Equipment Provided**

- -16 wooden sampling sticks
- -2 plastic 50mL sampling tubes in a Ziploc bag

### **Sample Method:**

#### **Bee Bread**

The samples must be comprised of fresh pollen or bee bread (depending on your interest) – not entombed pollen (pollen covered by propolis). The sample you collect should be from dry, grainy and sometimes colorful cells usually surrounding brood frames (see Figure 1). Avoid sampling bee bread that appears moist and dull in color unless you cannot find fresh pollen.



Figure 1: Fresh pollen on a frame

A minimum total of 3 grams of sample must be collected from 8 colonies sampled within the apiary. To collect 3 grams, it is advised to sample at least 4 full cells of comb from each of the 8 colonies. One tube should be filled with pollen collected from 8 healthy colonies and 1 tube should be taken from 8 weak or crashing colonies.

After you have taken the live bee sample from the brood area, look at the frames you have pulled to see if there are any cells with fresh pollen in them. If so, take one end of a sampling stick and insert it all the way to the bottom of the cell and rotate the stick all the way around the cell scraping the pollen as you go. You may damage adjacent cells as the stick is slightly larger than a cell (Figure 2) but this is normal. Avoid collecting wax and brood cocoon.



Figure 2: Opening cell wall to remove pollen

As you lift the stick from the cell, move slowly as the dry pollen is crumbly and may fall off the stick. Place the pollen or bee bread in the plastic 15ml. tube by scraping the stick on the inside mouth of the tube making sure that the pollen falls into the sampling tube. Repeat in at least 4-6 cells per colony to gather the minimum of 3 grams of pollen.

If the frame you removed for sampling does not have pollen in it, set it aside and try to find another frame with pollen in the colony. If you cannot find any pollen in the colony, move to another colony for sampling and try to get pollen from it. If you cannot gather pollen from a particular colony, it is necessary for you to take extra pollen from the remaining colonies to collect the requisite total of 3 grams. Each sampling stick will be used per each sample. The sampling sticks can be disposed of in the trash and should not be used again.

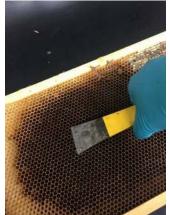
### Wax

Select a frame from the brood chamber that has drawn wax but little to no brood, nectar, or pollen. Find an area of the frame that has drawn wax but little to no brood, nectar, or pollen (Figure 3).



Figure 3: An empty frame ready for wax removal

Use the flat end of a clean hive tool to cut into the wax. Then, leaving one corner of the tool in place, pivot 90° (clockwise or counterclockwise) to cut out a quarter of a circle's worth of wax (Figures 4 and 5).



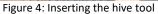




Figure 5: Wax removal with the hive tool

Using the hive tool and pick up the scraped piece of wax and place it into the 50-mL Falcon tube (Figures 6, 7 and 8).



Figure 6

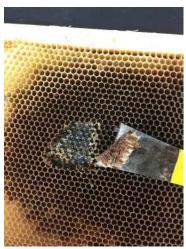


Figure 7



Figure 8

Repeat steps 4-8 for all 8 colonies, adding each piece to the same 50-mL tube. Use your gloved finger, if necessary, to push down the wax so all 8 pieces will fit and cap the tube. Cover the tube with aluminum foil to prevent sunlight from degrading any pesticides.

# **Shipping**

Once the pesticide samples have been collected from all colonies, place both tubes into a shipping box and send to the University of Maryland, College Park. Label the tubes with your name, date and colony type (weak or healthy) with an identifying number for those colonies sampled. The correct shipping address label has been included.

This sampling protocol is based on the USDA AHPIS National Honey Bee Survey. For additional information on this effort please <u>Wax Sampling Protocol</u>