Guidance for the Inspection of Alleged Cases of Pesticide-Related Bee Kill Incidents

Supplement to the FIFRA Inspection Manual

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Office of Enforcement and Compliance Assurance
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DISCLAIMER

This guidance represents the U.S. Environmental Protection Agency's (EPA)'s recommended procedures for conducting FIFRA compliance inspections. Deviations from this guidance on the part of any duly authorized official, inspector, or agent to follow its contents shall not be a defense in any enforcement action; nor shall deviation from this guidance constitute grounds for rendering the evidence obtained thereby inadmissible in a court of law. The mention of trade names or commercial products constitutes neither endorsement nor recommendation for use.

Purpose

This guidance was developed to identify unique considerations that state and federal inspectors should take into account when they are conducting Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) inspections as a result of a bee kill incident[1] related to honey bees and other social bees. These techniques may not apply to the inspection of bee kill incidents related to other bees (*e.g.* alfalfa leafcutting bees). This guidance will aid in standardizing bee kill inspections across federal, state and tribal agencies when trying to determine if the bee kill is related to the use of a pesticide in violation of FIFRA. The data gathered in these types of inspections will help determine if the bee kill was associated with the legal or illegal use of a pesticide. This guidance is intended as a supplement to the FIFRA Inspection Manual[2].

Bee Kill-Related Inspections

When conducting an inspection as a result of a bee kill incident, act promptly and follow a regimented plan that focuses on the circumstances of the incident as well as the collection of evidence that will be needed to prove any violations. All standard inspection and sampling protocols should be followed, including but not limited to issuing Notices of Use Inspection, creating and maintaining chains of custody for any samples collected for analysis, and issuing Receipts for Samples, as outlined in the FIFRA Inspection Manual. State or tribal pesticide inspectors should follow any agency or organization-specific use inspection and sampling protocols.

Bee kill-related inspections must focus on the immediate location of the bee kill incident as well as any surrounding areas in which pesticide applications may have occurred which may have influenced the incident. Three basic phases of determining the role of illegal pesticide use in a bee kill incident are as follows:

(1) The inspector should collect any additional information about the bee kill incident itself and plan and prepare for the inspection(s).
(2) The inspector should then inspect the affected hive(s) to collect observations and evidence related to the bee kill incident site.
(3) Finally, the inspector should identify and inspect sites of possible pesticide use in the areas surrounding or adjacent to the bee kill incident.

These "phases" of a bee kill-related inspection are discussed in more detail below. Upon completion of the inspection(s), the inspector should prepare and complete an inspection report, as outlined in Chapter 20 of the FIFRA Inspection Manual.

[1] Where pesticide applications result in the sudden (acute) mortality of adult forage bees or in the loss of entire colonies, the sudden mortality event is typically referred to as a "bee kill" incident. Incidents of Colony Collapse Disorder (CCD) are not included in the term "bee kill incident" and will not be inspected to determine pesticide use compliance as discussed in this guidance. A further discussion on CCD is found in Attachment III.
[2] http://www.epa.gov/oecaerth/resources/publications/monitoring/fifra/manuals/fiframanual.pdf.

Inspectors should be aware that the conduct of a particular bee kill-related inspection will be based on the specific facts of the incident itself. The phases described in this guidance are provided as a general inspection procedure; these phases may vary according to the specific circumstances of an individual incident. An example Bee Kill-Related Inspection Outline and an example On-Site Hive Inspection Checklist are attached in **Attachments I** and **V**, respectively, to aid inspectors in planning and conducting bee kill-related inspections. Inspectors are encouraged to tailor the inspection to the specific facts and circumstances of the incident.

Collection of Preliminary Information and Planning

Report of the Incident- Interviewing the Complainant

Bee kill incidents are typically reported to EPA or state and tribal pesticide program offices through a tip or complaint filed with the respective agency/program. Prior to initiating an inspection the inspector should interview the complainant to obtain pertinent incident-related information. In cases where the complainant does not have all of the information outlined below, the inspector should collect this information from the beekeeper during the on-site inspection of the apiary[3]. During the interview, the inspector should ascertain:

- the nature of the incident;
- where the incident occurred;
- when the incident occurred;
- the identity of any persons who may have been involved;
- why the complainant believes it happened; and
- why the complainant believes it was pesticide related.

The inspector should record the name of the person who reported the incident, with address, phone number and email address, and the date the incident was reported.[4] If the complainant does not self-identify for fear of retaliation or privacy concerns, this should be noted. The inspector should also determine if the complainant is the beekeeper of the affected hive(s).

The inspector should also record the date, time and nature (if known) of pesticide application(s) suspected by the complainant or otherwise potentially involved with the incident (*e.g.*, chemical name, product name, registration number, application rate, time of application, method of application, and targeted crop or site). If this information is not available, it should be noted in the report.

When possible, the exact location and nature of the incident should be described, including street addresses, Global Positioning System (GPS) coordinates and/or brief descriptions of the bee kill location that provide an understanding of the area or location where the bee kill event occurred. The inspector should describe whether and where dead bees are observed (either by the complainant or by the

[3] An apiary is a location where bees are kept and can include more than one colony; also known as a "bee yard."
[4] Any information which may be considered Personally Identifiable Information (PII) should be protected from release per the Privacy Act of 1974, 5 U.S.C. § 552a. See EPA's Privacy Act manual at http://www.epa.gov/privacy/policy/2190/index.htm.

inspector if the interview occurs during the on-site hive inspection) to document the claim that a bee kill incident has occurred. For a bee kill involving multiple colonies, the number and location of all affected colonies should be documented.

The inspector should also record, where possible, the actual or suspected date of the incident and/or dates over which adverse effects were observed. Given the large area over which colonies may be dispersed, it is possible that an inspector will not learn of a bee kill incident immediately. The inspector should interview the complainant to obtain a detailed description of how the bee kill incident was discovered. If the complainant cannot provide an estimate of when the event was initiated, it may be possible to gather information during the on-site inspection to provide better insight into the event (see On-Site Hive Inspection, below).

Pre-Inspection Planning

The following information should be collected (if available) prior to any physical inspection(s):

Location:[5] [6] If the exact location or an approximate address of the bee kill incident has been provided, the inspector should search for any publicly available information on the surrounding areas. Aerial photographs of the surrounding area may be available from an online source, such as Google© maps, to provide any possible locations of pesticide application in close proximity to affected colonies.

Weather Data: Information on weather data at or near the bee kill incident site, such as wind direction, at the time of pesticide application(s) and/or the bee kill incident may help determine where to begin looking for a suspected pesticide application. Use the closest and most accurate available source of weather data for determining weather conditions at the time of the bee kill incident or pesticide application. Recording information from three surrounding weather stations or collecting three National Weather Service station records would be most beneficial to analyze discrepancies.

Incident Reports: Section 6(a)(2) of FIFRA requires pesticide registrants to report known adverse effects associated with their chemical(s) (including incidents such as bee kills) to the EPA. Although non-registrants are not required under FIFRA to report ecological incident data, they have the ability to do so directly to EPA or through the National Pesticide Information Center (NPIC[7]) via the Ecological Pesticide Incident Reporting web portal[8] or via telephone, FAX, or e-mail[9]. All ecological incidents reported

[5] Depending on the state in which the bee kill incident occurred, the inspector may be able to identify crops in adjacent areas using Driftwatch. http://www.driftwatch.org/

[6] The inspector may reference a Registered Apiary List for information on additional apiaries nearby which may have been affected, if such a list is maintained for the state in which the bee kill incident occurred.

[7] http://pi.ace.orst.edu/erep/

[8] USEPA. 2012. Pesticides: Environmental Effects. Pollinator Protection. Report a Bee Incident portal. http://www.epa.gov/opp00001/ecosystem/pollinator/

through NPIC, including incidents involving bee toxicity, are supplied to the Office of Pesticide Programs (OPP), EPA Headquarters, on a regular basis and by request. Upon receipt of a report on an ecological incident, OPP creates an Ecological Incident Report which is verified, if possible, with final inspection reports. This information is used to help support risk conclusions and help flag chemicals of ecological concern. If a bee kill has been reported to EPA, the inspector may obtain a copy of the Ecological Incident Report. This can be done by submitting a request for an Ecological Incident Information System report to EPA.

The inspector should collect and inspect all needed personal protective equipment (PPE) to ensure his/her safety during the inspection. Chapter 16 of the FIFRA Inspection Manual provides general guidelines for inspector safety and PPE. The inspector should also determine if any bee hive inspection-specific PPE is needed. This may include, but is not limited to:

- Protective clothing (long sleeve shirt, long pants, protective shoes, elastic gathers to secure pant legs, hat, bee veil, bee suit, gloves, *etc.*);
- First Aid kit, including any necessary emergency allergy medicine such as an EpiPen®.

Additionally, the inspector should have a sampling plan in place and determine the sampling equipment needed to ensure proper collection of physical samples throughout the inspection. Chapter 13 of the FIFRA Inspection Manual provides some general recommendations on sampling equipment; the inspector should consult the analyzing laboratory with any specific questions regarding the type of sampling equipment needed for a specific sample to be collected (*e.g.,* the sample jar or bag size needed for a specific sample) as well as guidelines for size or quantity of sample to be collected. Any samples collected as part of a bee kill-related inspection should be collected in accordance with Chapter 13 of the FIFRA Inspection Manual. (See also **Attachment II** for a discussion on bee kill incident-related sampling.) If a state/tribe apiary inspector is available, make arrangements to have them assist in the inspection and sample collection.

On-Site Hive Inspection

Interviewing the Beekeeper

Prior to inspecting the hive(s) involved in the incident, the inspector should interview the beekeeper to determine if the colony (or colonies) was (or were) completely killed or only partially affected and whether it is recovering. The inspector should document the date and time of the last inspection of the hive(s) by the beekeeper or other knowledgeable persons[10] and collect a copy of the hive inspection report, if available. The inspector should also record any feeding done by the beekeeper including information on the timing and frequency of feeding and watering and identification of the type of food provided by the beekeeper. If the incident occurred in a research apiary, the inspector should describe whether the incident was associated with any ongoing studies or research.

[9] beekill@epa.gov

[10] This may include inspections conducted by the state apiarist, if required by State law.

The inspector should obtain all information on pesticide applications to the hives in question made by the beekeeper or other person(s) in the last year, documenting the date, time and nature (if known) of pesticide application(s) to the hives (*e.g.*, chemical name, product name, application rate, time of application, method of application, name of the applicator and, if a restricted use pesticide (RUP), the name of the certified applicator). The following information should be collected, for each pesticide which was applied to the hive:

- Product Name;
- EPA Registration Number;
- Active Ingredient(s);
- EPA Establishment Number;
- Distributor Name(s) and Address(es);
- Date of Application;
- Method of Application; and
- Target Pest.

If this information is not available, it should be so noted in the inspection report. The inspector should also collect labels or copies of labels for all products used by the beekeeper for pest control within the last three (3) months at minimum, ideally including labels applied within the last year (Follow the FIFRA Inspection Manual protocol in Chapters 9 for documentary sample collection.) If misuse[11] of a pesticide(s) is suspected, include the name, address, and phone number of the distributor where the product(s) were purchased. Collect a copy of the receipt documenting that purchase, if available. If the use of a registered pesticide is suspected, it may be necessary to collect a physical sample of the product to determine if that product was formulated properly. Collection of physical samples of pesticides must be completed in accordance with the protocol outlined in Chapter 9 of the FIFRA Inspection Manual.

The inspector should follow inspection protocols described in Chapter 12 of the FIFRA Inspection Manual to gain consent from the beekeeper to conduct the interview and subsequent inspection of the hive or bee kill site. If the beekeeper does not provide consent, the inspector may be able to obtain a warrant.

Inspecting the Hive and Bee Kill Site

For the safety of the inspector and the hive, in-hive inspections should **NOT** be attempted by an inspector if the inspector does not have experience with handling bee colonies. Bees, hives, frames, *etc.* must be handled by the beekeeper, an accompanying state apiarist, or an inspector with knowledge of bee colonies and/or beekeeping training. Inspectors should be properly dressed with bee protective clothing/attire to minimize the risk of bee stings regardless of whether they personally handle a hive.

[11] "Misuse" refers to the use of a pesticide in a manner inconsistent with its labeling, Section 2(ee) of FIFRA.

The inspector should collect samples[12] of dead and dying bees from outside the hive entrance. If the bee kill incident occurred away from the hives, the inspector should visit and inspect this area as well, collecting any samples (*e.g.,* dead bees, vegetation, soil, or water) as needed and available. Ideally, samples of bees should include bees that appear to be dying or to have died recently and are not in advanced stage of decay. Evidence or stages of decay may provide an estimation of when the bee kill incident occurred, if it is unknown to the beekeeper. The inspector should document any signs of decay by photographs and by noting any observations. (See **Attachment III** for signs of bee decay and a timeline of their occurrence). Bees that appear to be acting abnormally (*e.g.*, ataxia, lethargy, jerking movements) should also be sampled. If drift or a direct overspray of pesticides is suspected or if the source of exposure is uncertain, then swab/wipe samples should be collected from the exterior surface of the hive. Follow standard pesticide residue sampling procedures when collecting surface samples.[13] The size (quantity) of the samples needed depends on the analytical method that will be used for analysis.

Depending upon the facts of the incident, it may be appropriate to collect additional samples from the interior of the hive. These might include samples of stored pollen, wax, honey, or other items from inside the hive. Samples of syrup, water or other substances used to nourish the bees may also be collected. See **Attachment III** for additional information on hive dynamics and possible samples to be collected within hives.

Note: If the inspector is not trained to handle bees, the beekeeper may take samples on the inspector's behalf, provided samples are taken in the presence and under the direction of the inspector. The identity of any third party collecting samples must be documented in the inspection report. Photographs should be collected to document inspection activities and observations made during the inspection of the apiaries. The inspector must assure that third party collectors collect samples within the appropriate sampling procedures and protocol to protect the integrity of the sample.[14]

When inspecting the impacted bee hives, the inspector should be observant and note anything that might suggest the involvement of a pesticide. Include observations concerning the general condition of the impacted hive(s) and other nearby hives. Note any anomalies of the hives or the bees inside the hives. **Attachment III** can provide some insight as to other abnormal conditions that may affect a bee colony. The inspection report should document if any such conditions exist so that these factors can be considered in determining if a pesticide was involved in the incident.

[12] All sampling should be conducted in accordance with the inspecting agency's sampling Standard Operating Procedure.

[13] See Chapter 13 of the FIFRA Inspection Manual.

[14] This includes, but is not limited to, such practices as wearing disposable gloves when collecting samples to prevent cross-contamination.

Identifying and Inspecting Potential Pesticide Sources

To evaluate the potential role of a pesticide in a reported bee kill incident, the inspector should focus first on sites at or immediately adjacent to the incident location at a distance within sight range[15] and sites where information provided suggests the source of pesticides may be. If no potential sources of pesticide are identified through the inspector's observations at the bee kill incident site or through interview of the complainant and/or the beekeeper, the inspector should first evaluate the locations closest to the incident for the likelihood of concurrent pesticide presence. The inspector should note sites in which prior pesticide exposure to bees was possible, including, but not limited to:

- Sites where crops[16] are frequently sprayed or have been known to have received a recent application;
- Sites where pesticide treated seeds have recently been planted;
- Areas or sites with flowering plants (crops, weeds, ornamentals) or other plants which bees might consider desirable for foraging;[17]
- Rights-of-way such as utility lines or roadside drainage ditches.

Knowledge of the surrounding area, including any influencing weather patterns during the bee kill incident, will aid the inspector in identifying sites to inspect. (For example, if the incident occurred on a windy day, the inspector may want to first focus on areas which were upwind of the bee kill incident.) Additionally, the inspector may take crop or site descriptions into account to identify potential sources or sites of the affected colony's exposure to pesticides. Inspectors may also take into account any locations of treated seed planting when identifying locations of potential pesticide sources. Note: Treated seed (and any resulting dust-off from treated seed) itself may be exempted from registration under FIFRA[18] as a treated article and as such its planting is not considered a "pesticide use." However, if the inspector suspects or has reason to believe a treated seed is subject to registration (*i.e.*, the seed is

[15] Generally, "within sight range" is considered to be in the range of between ¼ and one mile, although this could vary in some cases.

[16] If such information is available, the inspector may consider whether a particular crop is considered pollinator dependent (*i.e.*, requires insect pollination rather than wind pollination), pollinator attractive (*i.e.*, good source of pollen and/or nectar) or not expected to attract pollinators. For crops that may not be pollinator attractive, recognize that bees may not have been attracted to a particular crop but rather may have been attracted to plants/weeds that are blooming in the vicinity of the crop. The presence of flowers alone does not determine the attractiveness of a crop to bees as some plants (*e.g.*, cotton, sweet cherries) have extra-floral nectaries that are attractive to bees as a source of nectar. Also, bees can be exposed to pesticides through consumption of drinking water (puddles in treated fields; irrigation water) that they may use to cool the colony and/or dilute honey prior to consumption.

[17] There are several online resources that may help the inspector in determining which plants are "desirable" for bees:

- USDA listing of native pollinator attractive plants: http://www.ars.usda.gov/Main/docs.htm?docid=12052;
- Xerces Society Regional listings of plants: http://www.xerces.org/pollinator-resource-center/ and
- Pollinator Partnership app for identifying pollinator-attractive plants by locale: https://play.google.com/store/apps/details?id=com.pollinator.gardening.

[18] 40 C.F.R. § 152.25(a).

not in compliance with the treated article exemption), plantings of that treated seed may nonetheless be investigated. (See Appendix E of the FIFRA Inspection Manual for further considerations).

If no obvious source is apparent from the inspector's observation, it may be necessary to expand the search area for a source. In particular, vector control activity (such as application of a mosquito adulticide) in the area could be a factor and such use may not be apparent to the inspector without checking with the local or state vector control office. If mosquito control activity has occurred, it may also be necessary to determine if a public health emergency had been declared due to detection of mosquito-borne diseases or pathogen-carrying mosquitoes in the area.

Once possible sources have been identified, it may be necessary to enter upon that property to conduct an inspection to determine if use of a pesticide(s) at that site was responsible for the bee kill incident.

Conducting Pesticide Inspections

Inspectors must follow the inspection protocol detailed in Chapter 12 of the FIFRA Inspection Manual when investigating any potential misuse of pesticides related to the bee kill incident. For any pesticide applications made in the area at the time of the bee kill incident, include all of the information listed in the Inspecting the Hive and Bee Kill Site section, above, for each pesticide application as well as the application method and rate of application.

Documentary evidence should include pesticide application records for suspect treated areas. Documentary samples of the labels for products used along with a statement from the applicator that the label is a true and accurate representation of the pesticide used should be obtained. Follow the Inspection Manual protocol to properly obtain a documentary sample. If product contamination or improper formulation is suspected, a physical formulation sample of the pesticide should be collected.

In cases where drift or direct overspray is suspected, it may be necessary to collect vegetation samples or other residue samples to document off-site movement of the pesticide. Refer to the Inspection Manual for the proper protocol for sampling different types of residue media. Inspectors should ensure that all samples obtained are properly collected and documented, and that a Receipt for Samples is properly signed by the owner of the property where the sample was collected.

~~ATTACHMENT I~~

Example Bee Kill-Related Inspection Outline

Preliminary Information Collection and Planning:

1) Interview the Complainant:
   a) Basic Information
      i) Name of the person who reported the incident, with address, phone number, and e-mail address [Personally Identifiable Information should be safeguarded from release].
         (a) Note whether the complainant is also the beekeeper of the affected hive(s).
      ii) Date the incident was reported.
   b) Incident Description
      i) Location of the incident (as specific as possible; including GPS coordinates, if available).
      ii) Date(s) of the incident.
      iii) Details about the site where the incident occurred. Describe the land use of the area where dead bees were observed including farm, orchard, home, and any unusual features, such as proximity to a shelter belt of trees, or other observations that will provide understanding of the area surrounding the bee kill-incident. Photograph relevant areas. Date, time and nature of suspected pesticide application(s):
         (1) Chemical name,
         (2) Product name,
         (3) EPA registration number,
         (4) Application rate,
         (5) Any non-pesticidal ingredients (adjuvants, surfactants, fertilizers, *etc.*) used in the application
         (6) Time of application,
         (7) Method of application,
         (8) Location of application, and
         (9) Identity of any persons involved in application.
      iv) Identity of any person(s) the complainant suspects of intentionally causing the bee kill.
      v) Collect a description of the type of incident, including:
         (1) Symptoms or adverse effects, if known by the complainant,
         (2) Estimate of the number of bees killed or adversely affected,
         (3) The number of hives that were affected/not affected, and
         (4) The proportion of bees in each hive that were affected.
         (5) Details on bee species affected in the bee kill incident should include species name(s).
      vi) If other organisms were also affected, include a list of the organisms that were observed to be affected (*e.g.*, birds, fish, mammals, reptiles, plants, trees, other).
         (1) Number of affected species.
         (2) Observed symptoms or effects, such as death, ataxia, lethargy, spasms, discoloration of surrounding leaves, wilting leaves, *etc.*
2) Pre-Inspection Planning:
   a) Collect information regarding the area surrounding the bee kill incident
      i) Identify any areas (fields, orchards, *etc.*) or other such locations where pesticide applications are likely to have occurred.
      ii) Identify any areas or locations suspected by the complainant to have affected the bee kill incident.
      iii) Determine if other beekeepers have hives near the bee kill incident.
         (1) If neighboring hives are affected, expand investigation.
   b) Collect weather data for the location and time of the bee kill incident.

c) Collect copies of relevant incident report(s) submitted to EPA.
d) Review inspection and sampling plans/procedures.
e) Gather necessary inspection or and sampling equipment.
f) Gather and inspect necessary PPE.

On-Site Hive Inspection:

3) Interview the beekeeper:
   a) Collect date and time of last inspection of the hive(s) by the beekeeper, state apiarist or knowledgeable person.
      i) (Collect the report from this inspection).
   b) Collect relevant information on the general health of the affected hive(s) if inspected.
      i) Note strength of colony, bee behavior, brood pattern, queen rightness, condition of bees.
      ii) Note signs of disease, parasites, *etc.*
      iii) Note signs and collect records of active treatment.
      iv) Note any management techniques implemented in the last 3 weeks (examples: colony moved, split, "requeened," *etc.*).
   c) For commercial colonies, document:
      i) Where the colonies were located prior to the alleged bee kill.
      ii) When the colonies were moved to the location of the alleged bee kill.
      iii) Whether the colonies were "in transit" prior to the alleged bee kill.
      iv) Whether honey was removed from the colonies prior to transit, and
      v) Whether the colonies were inspected by a state or other independent apiarist prior to being moved into the location of the alleged bee kill. Determine whether there is a written report from that inspection, and collect a copy if available.
   d) Collect information on any feeding/watering done by the beekeeper.
   e) Collect information on all products applied to the hives in question made by beekeeper or other person(s) in the last year, including:
      i) Product Name;
      ii) EPA Registration Number;
      iii) Active Ingredient(s);
      iv) EPA Establishment Number and
      v) Distributor Name(s) and Address(es).
   f) Collect labels (or copies of labels) of pesticides applied in the hives within the last 3 months.
   g) Collect distributor name, address, and phone number as well as copies of any receipts of distribution or sale of any pesticides suspected to be involved in misuse.
      i) Conduct follow-up inspection(s) per Chapter 12 of FIFRA Inspection Manual, as needed.
   h) Collect any physical samples of pesticides to verify formulation.
4) Inspect and collect samples from the hive(s):
   a) Note the location of all dead bees; acute mortality events from pesticides typically result in a pile of dead bees at or near the entrance of the hive.
   b) Photograph hive and bees, including any dead bees, as well as area surrounding hive/incident.
   c) Collect samples of dead bees and/or any bees which are acting abnormally.
      i) Note or describe any "abnormal" behavior observed.
   d) Collect sample of honey and honeycomb if a hive treatment product is suspected or known to have been used.
   e) Take swab samples for the hive exterior to determine if drift has occurred.
   f) Take vegetation samples around area of hive(s) (if applicable).

g) Collect sample of syrup or other bee food /water provided to the hive(s). Collect information on timing and frequency of bee food and water provided by the bee keeper.

h) Clearly identify and note any samples which were collected by on the inspector's behalf. Samples must be collected when the inspector is present. Document sampling process, including use of appropriate PPE.

5) Inspect and collect samples (as outlined in 4 above) from the bee kill incident site (if in a different location than the current location of the hive).

Identifying and Inspecting Potential Pesticide Sources:

6) Identify Potential Pesticide Sources:
   a) Identify pesticide application sites, including plantings of treated seed, adjacent to or near affected hive(s) or bee kill incident site(s).
   b) If pesticide source unknown, identify probable locations of pesticide application in the area surrounding the bee kill incident, including:
      i) Sites suspected by the complainant and/or beekeeper.
      ii) Sites containing crops, orchards, fields, *etc.* which may have been attractive to the affected colony prior to the bee kill incident.

7) Conduct Inspection(s):
   a) Follow all pesticide inspection protocol(s) including presentation of credentials, issuance of Notices of Inspection, *etc.*
   b) For any pesticide applications made at the time of the bee kill incident, collect or document:
      i) Date and time of pesticide application(s).
      ii) Distance from the treatment site where dead or adversely affected bees were found, or from the location of the affected hive(s) to the nearest site were pesticides were applied.
      iii) Pesticide information:
         (1) Product name(s),
         (2) EPA registration numbers,
         (3) Active ingredient(s),
         (4) EPA producing establishment registration number(s),
         (5) Distributor name(s) and address(es), and
         (6) Copies or photographs of pesticide labels and labeling.
      iv) Application method used by the applicator (*e.g.*, aerial, ground, chemigation).
      v) Document type of application equipment used (*e.g.*, brand, model) used for the pesticide application and where possible the last calibration, nozzle type, nozzle spacing, screens, filters and pressure (functioning gauge).
      vi) For planting of treated seed, document:
         (1) Whether the seeding equipment was pneumatic,
         (2) The identity of the product, including product name and EPA Reg. No., used to treat the seed, if available,
         (3) A copy of any label for the treated seed, if applicable, and
         (4) If the treated seed is suspected not to meet the exemption at 40 C.F.R. § 152.25(a), document the date of purchase and the identity of the distributor of the treated seed, collecting any copies of sale or distribution records as available.
      vii) Provide the amount of pesticide applied (in lb a.i./acre or amount of product/acre).
      viii) Information on the crop treated (name of crop, information on whether or not the crop was in bloom during application, *etc.*).
      ix) Certification credentials of the pesticide applicator.
      x) If appropriate, collect physical samples (formulation samples, vegetation, residue, *etc.*).

Sample Collection

Chapter 13 of the FIFRA Inspection Manual "was developed to aid field personnel in the collection of samples during pesticide incident inspections."[1] Follow these guidelines for sample collection. This document provides additional considerations to incorporate when planning a bee kill incident-related inspection.

Sampling plans developed for bee kill-related inspections should consider the need to both demonstrate whether a pesticide is present in affected colonies, and determine how it got there and from where it originated. This can be accomplished through the collection of physical samples at the bee kill incident site, at the location of the affected hive and in areas adjacent to those locations. The sample plan should consider all potential samples (*e.g.*, vegetation, soil, water, *etc.*) which may be necessary to determine a pesticide use and its relation to the bee kill incident.

Inspectors should consult the analyzing laboratory prior to sample collection to determine the needed sample size. If additional factors discovered in the field affect the planned sample collection, the inspector should contact the laboratory during the inspection to seek guidance and/or clarification. Samples may be split with the beekeeper, if requested and feasible. Consideration must be given to whether there is a sufficient sample to enable analysis; splitting samples can result in an insufficient sample size where no analysis may be possible.

Bees

Analysis of bees is typically done by grinding a composite sample of bees. This type of sample may be collected to determine a possible pesticide exposure. Inspectors should be aware that due to this process, the results of this analysis will not provide an indication of the route of exposure (*e.g.,* ingestion or dermal exposure) which resulted in a pesticide residue. To determine how a bee hive or colony was exposed to a particular pesticide, the inspector must rely on additional observations or sample collection from the hive, bee kill incident site, areas adjacent to the bee hive, *etc.*

To aid in determining whether a bee kill incident occurred as a result of pesticide use, it is important to collect samples of bees from the affected hive(s). Dead and dying bees from outside the hive entrance should be collected. Ideally, samples of dead bees should include bees that appear to have died recently and are not in advanced stage of decay (See **Attachment III** for signs of bee decay). Bees that appear to be acting abnormally (*e.g.*, ataxia, lethargy, jerking movements) should be sampled as well. Samples of dead bees and impaired bees may be composited in a 250-ml glass jar.

Live bees which appear to be acting "normally" may also be sampled to ensure a proper sample size is collected and to determine whether a pesticide residue is present. Inspectors should take care not combine samples of live and dead/dying bees unless otherwise directed by the analyzing laboratory. For the safety of the inspector and the hive, the beekeeper may be consulted for direction or assistance in collecting samples of live bees. Bees that are still alive should be quickly frozen. Some pesticides can

[1] Page 13-1, Chapter 13, FIFRA Inspection Manual, February 2002.

degrade quickly in the environment; therefore, it is imperative that all samples, including bees, be collected as quickly after the incident as possible.

The size (quantity) of the samples needed depends on the analytical method that will be used for analysis. Chapter 13 of the FIFRA Inspection Manual recommends collecting a minimum of four ounces of honeybees per sample; however, the inspector should consult with the analyzing laboratory for a specific sample size when collecting bees. Bees may lose more than 90% of their body weight once they have died; accordingly, samples of dead bees may require the collection of a larger number of carcasses compared to samples of live, dying, or recently deceased bees. When determining the number of bees required for an individual sample, inspectors may find the following estimations (based on a 10 gram sample size) helpful:

- 100 live, dying, or newly dead bees;
- 1,000 dead and desiccated bees.

Residue

If drift or a direct overspray of pesticides into the apiary is suspected or if the source of exposure is uncertain, then swab (wipe) samples should be collected from the exterior surface of the hive(s). Follow standard pesticide residue sampling procedures found in Chapter 13 of the FIFRA Inspection Manual when collecting surface samples. If there are a large number of colonies that appear to have been affected in an apiary, a systematic sampling scheme may be necessary. If resources are limited, the inspector should select the colony with the largest number of dead bees either in front of or within the colony.

Vegetation

Blossoms (including pollen and nectar) may be sampled as a source of exposure to a pesticide applied approximately at or before the bee kill incident. Sampling foliage where a bee might land is less valuable in determining a source of pesticide exposure to the bee than sampling a plant bloom where bees are likely to land. Collect samples from sites that are attractive to bees, where possible. One means of doing this is for the inspector to look for pollen and nectar sources in the area and then look at areas where pesticides may have been applied, such as flowering weeds along or within treated fields. Vegetation samples may include a progressive set of samples from the apiary or site of possible pesticide exposure (*i.e.,* a location or flowering plant(s) attractive to bees) to the point of application to document drift. See Chapter 13 of the FIFRA Inspection Manual for a detailed discussion of progressive or gradient sampling.

If drift of pesticides is suspected, vegetation samples should be collected from plants growing in the suspected treated area, as well as other plants between the treated area, areas of attractiveness to bees, and the impacted hive(s). In this case, samples may consist of whatever plant surface may have been exposed to the pesticide application or the drift from that treatment. Vegetation in each different sampled area should be kept separate and not composited so that the analytical results can track the movement of spray material off-site towards the hive(s).

Samples within a Hive or Colony

Some knowledge of hive dynamics is important in order to take a proper sample. Inspectors not trained in hive dynamics, or unsure of the proper location within a hive for sample collection, should consult the beekeeper, state/tribal apiarist, or other knowledgeable person with any specific questions about the sampled hive.

It is preferable to collect individual samples from hives, but it may be necessary to collect composite samples from multiple hives in order to achieve sufficient sample size or prevent further stress to colony health.

Colony matrices that may be sampled include wax, pollen, honey and brood, as needed. Depending on the colony age, wax and comb pollen samples may contain a number of pesticide residues. Surveys of managed migratory bee colonies indicate that a broad range of pesticides have been detected in hive products (*e.g.*, honey, stored pollen, wax). The most frequently detected pesticides and the two that occur in the highest quantity are those used by beekeepers to control varroa mite (*i.e.*, coumaphos and fluvalinate). Typically, a combination of pesticides is detected in the same hive matrices. Honey samples should be collected from fresh honey in the top of the hive and pollen samples should be collected from uncapped (*i.e.*, recently collected) pollen chamber near the brood chamber.[2] Brood chamber, wax and other areas of the hive may contain residues collected over time.

When sampling pollen and/or honey from comb, care should be taken not to include wax since wax can contain a different spectrum of pesticides than what may actually be present in pollen or honey. Brood wax is generally dark brown to black. Honey wax is pale and light colored.

Keep in mind that when sampling pollen from comb, bees do not typically store pollen in unique batches. Pollen collected from a number of floral sources over time may be stored in the same cell of the comb. The comb area sampled across colonies should be relatively consistent (*e.g.*, from frames outside of the brood areas (area without larvae) since the inspection should not impact remaining brood survival if at all possible).

Honey and honeycomb should be sampled if the inspector suspects that a pesticide has affected the hive(s). In-hive materials that might be used as applicators for miticides and/or other pesticides used within the hive *etc.*, should also be sampled. If the hives are being fed dietary supplements such as sugar syrup, such materials should be sampled.

Soil

Soil samples should be taken if a soil applied insecticide has been used/or treated seed has been planted in the field alleged to have caused the bee kill. If it is suspected that illegal use of treated seed is a causal factor where soil became contaminated through dust-off or excessive leaching of treated seed coatings,

[2] Older stored pollen is often capped with wax or "entombed".

information related to seed treatment should be obtained, where possible. If custom seed treatment has occurred, it may be possible to obtain information related to specific pesticides applied to the seed.

Water Samples

Water samples may be collected in connection with bee kill incidents, especially when the potential pesticide source is a mosquito larvicide. Water samples taken are usually from roadside ditches, irrigation ditches, or other small bodies of standing or stagnant water where vector control applications may have been made. Refer to Chapter 13 of the Inspection Manual for appropriate sampling protocol and quantities needed for an adequate analysis. Collection of water from chemigation devices should also be considered.

Sample Handling

When handling pollen, honey or bee samples, it is important to slow decay of the samples caused by bacteria and/or fungi as quickly as possible. Immediately cool the sample using ice or storage in a freezer and transport to laboratory for analysis as quickly as possible. If longer-term storage (*i.e.*, longer than 1 – 2 days) is necessary, samples must be frozen at or below -18^{o}C (0^{o}F).

If soil or water samples are collected, they should be handled, stored, and shipped per Chapter 13 of the FIFRA Inspection Manual or according to the guidance of the analyzing laboratory.

Sample Storage

In order to ensure the stability of pesticide residues in biological samples collected during an inspection, the samples should be protected from light to the extent possible and should be placed either on ice or dry ice once they have been properly labeled. See Chapter 13 of the FIFRA Inspection Manual for specific guidelines on sample storage, preparation, and shipping.

Analysis

Depending on the extent to which the suspected cause of the incident may be known, residue analysis may be broad spectrum (multi-residue) or relatively focused examining specific active ingredients and their degradates (transformation products). Although most state lead agencies have established protocols for conducting residue analysis, an approach has been identified by the U.S. Department of Agriculture Agricultural Research Service that provides a relatively inexpensive process for multi-residue analyses of biological samples. This approach goes by the acronym QuEChERS[3] (standing for "Quick, Easy, Cheap, Effective, Rugged, and Safe") and as the name suggests, it provides a rapid means of conducting multi-residue analyses.

It may be possible to ascertain where bees have been foraging through an analysis of the pollen. Referred to as a palynology (*i.e.*, the study of dusts or of things that are strewn), pollen from forage bees or within the comb can be examined microscopically. Pollens from different plants have different colors, textures/shapes that can be used to determine their floral origin.

[3] To learn more about the USDA QuEChERS approach, refer to:
http://www.ars.usda.gov/is/ar/archive/jul03/quech0703.htm

Sampling and Sample Analysis

In addition to the wide variety of residue samples to be analyzed, samples can be analyzed to verify formulation to determine possible product contamination or to verify the contents of a tank mix. These samples will be analyzed by a formulation chemistry laboratory.

Coordination with the Laboratory

Prior to conducting a bee kill-related inspection, the inspector should contact the laboratory that will analyze any physical samples collected. The inspector should ensure that 1) the sample collection planned for the inspection and 2) any sample collection and preservation supplies/equipment planned to be used are consistent with the laboratory's standard operating procedures. Although Chapters 9 and 13 of the FIFRA Inspection Manual provide guidelines for the methods of sampling and types of sampling equipment that may be used in the collection of pesticide and environmental and residue samples, the inspector should defer to the laboratory for any specific sample size, equipment, or collection methods needed for obtaining samples.

The inspector and laboratory should also discuss what pesticide(s) or active ingredient(s), if known or suspected by the complainant or inspector, was involved with the bee kill incident. Inspectors should discuss whether the laboratory will be able to complete the analysis of any samples collected for that particular pesticide or active ingredient with their available equipment, standards, and methods. The QuEChERS approach, and variations upon that approach, can be used by laboratories when:

- A particular pesticide(s) is/are suspected;
- Selecting a method for pesticide analysis in an atypical matrix (*e.g.*, wax, pollen, nectar or honey);
- Sample weight is limited;
- The laboratory's equipment is limited; and
- When a laboratory cannot achieve the method detection levels pertinent to the data quality objectives of investigation with their own methods.

If a specific pesticide product(s), active ingredient(s), pesticide degradate(s) or products containing pesticides (*e.g.*, treated seeds) is not known or suspected to be related to the bee kill, then the inspector should discuss this with the laboratory as well. A general screen can be performed on samples collected from the hive or colony (honey, wax, bees, *etc.*) to help determine whether the hive has been exposed to a particular pesticide or pesticides. The inspector should be aware of the laboratory's ability or limitations in performing such an analysis and should discuss which analytes are available to the laboratory for inclusion in any analysis.

This attachment discusses attributes of bees and their colonies that should be considered when investigating bee kill incidents. Familiarity with bees and their biology and patterns of behavior, as well as apicultural practices, can provide important insight into understanding situations an inspector might observe during a bee kill investigation.

Beekeeping

It is not the intent of this guidance to provide inspectors with expertise in beekeeping; rather, the intent is to provide inspectors with an understanding of things to consider when investigating the loss or impairment of bees or bee colonies that has been may be linked to the use of a pesticide. If inspectors are interested in learning more about beekeeping, there are opportunities to do so through local beekeeping organizations[1], through university extension services[2] and through on-line resources[3] as well as working with and instruction from a state apiary inspector. Such sources of information are not substitutes for actual hands-on experience with bee colonies. Inspectors should **NOT** attempt to manipulate a honey bee colony without sufficient practical experience.

The size of beekeeping operations can vary considerably. The size of the beekeeping operation will dictate whether colonies are permanently located in a particular spot (fixed) or whether they are moved around (migratory) to support pollination services and/or honey production.

The number of colonies positioned in a particular area will be dictated by available forage; however, some of the larger beekeepers may place their colonies in temporary locations (*i.e.*, holding yards) when colonies are moved to differing locations. Migratory colonies may be located on wooden pallets to facilitate transport or to ready colonies for deployment to pollination locations; these colonies also tend to be of relatively uniform dimensions in order to facilitate stacking during transport. For colonies involved in honey production, the number of "supers"[4] on the colony is based on the ability of that colony to produce honey.

Bee Kill Incidents

Bee kill incidents typically focus on honey bees (*Apis mellifera*) for a variety of reasons (*e.g.*, they are social insects and exposure to pesticides can result in the loss of large number of adult bees; colonies are monitored and managed by beekeepers, which increases the likelihood of detecting adverse effects; and honey bees are a valuable commodity which increases the motivation to report suspected pesticide

[1] For a listing of state honey and beekeeping associations, see: http://www.honeyo.com/org-US_State.shtml.
[2] Beekeeping 101 is an example of an on-line beekeeping course (fee for service) that is offered by Pennsylvania State University (http://beekeeping101.psu.edu/).
[3] An example of an on-line resource is Beekeeing Basics (ftp://ftp.fao.org/docrep/fao/012/a0849e/a0849e00.pdf) that has been made available through the Mid-Atlantic Apiculture Research and Extension Service.
[4] For "surplus" honey production, these are sections of the hive the bees fill with honey to provide a "surplus" of honey beyond that needed for the bees to live on and rear replacements. Graham, et. al., 1992. The Hive and the Honey Bee, page 541.

incidents). Given that honey bees can forage for up to 7 kilometers (4.3 miles)[5], the potential routes of exposure can encompass a relatively broad area. Additionally, bee kill incidents can result from other factors in addition to pesticides and these potential factors should be evaluated and accounted for during any bee kill investigation. Therefore, inspections of bee kill incidents require some knowledge of bee colonies[6] and the factors that can influence the survival of both individual bees and the colony on which they depend.

Colony Collapse Disorder

Because colony losses can be attributed to a variety of causes, it is important to be able to differentiate whether the loss is a result of illegal pesticide use or whether it may be due to other causes.[7]

In 2006 beekeepers in the United States began discovering colonies characterized by the disappearance of adult worker bees with few or no dead bees in the colony, the presence of capped brood with a small cluster of nurse bees, the queen, and the presence of intact honey and pollen stores. This syndrome has been termed Colony Collapse Disorder (CCD)[8] (since the absence of the colony's forage bees coupled with a very limited number of in-hive bees to care for developing brood results in the death, *i.e.*, collapse, of the remainder of the colony). Colonies suffering from CCD did not exhibit any evidence of large numbers of dead bees in the vicinity of the colony that could account for the relatively sudden loss of the colony's forage bees. Although a number of factors have been associated with CCD including honey bee pests (*e.g.*, varroa mite[9]), diseases (*e.g.*, the fungal disease Nosema[10]), poor nutrition due to inadequate forage, and pesticides, none of these factors have in and of themselves been identified as a cause. The available information indicates that CCD results from a combination of factors; however, the

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

[6] A wealth of information on the structure and function of honey bee colonies is available through the internet. Agricultural Extension websites such as the Mid-Atlantic Apiculture Research and Extension Consortium (https://agdev.anr.udel.edu/maarec/honey-bee-biology/the-colony-and-its-organization/) can provide useful information on the bee colonies as well as regionally specific information on bee pests and pathogens. State Apiarists can also provide additional information about bee hive health.

[7] See also the discussion of late season death of drones in the Subsection titled *Bees can die on their way to and from the colony at the entrance to the colony and within the colony* under the Challenges for Investigating Bee Kill Incidents.

[8] USDA. 2007. Colony Collapse Disorder Action Plan. CCD Steering Committee. June 20, 2007. http://www.ars.usda.gov/is/br/ccd/ccd_actionplan.pdf

[9] Varroa mites (*Varroa destructor*) represent a serious external parasite of bees; the mite feeds on the hemolymph (blood) of both adult and larval/pupal bees and can lead to the death of individual bees and the serious weakening of the colony. Many agricultural research and extension service publications provide information on the detection and treatment of varroa mites (*e.g.*, http://agdev.anr.udel.edu/maarec/wp-content/uploads/2010/03/Varroa_Mites_PMP2.pdf). Because of the severe effect that this pest can have on honey bee colonies not just through its direct effect as a parasite but also because it can carry (vector) bee diseases, beekeepers have gone to considerable lengths to combat varroa mites and in many cases have relied on pesticides, i.e., varroacides, to kill in-hive mites.

[10] Nosema is a fungal disease that affects the midgut of bees and is caused by the spore-forming *Nosema ceranae* or *N. apis* microsporidian fungus. Agricultural research and extension service publications provide information on the detection and treatment of Nosema (*e.g.*, http://www.extension.org/pages/27064/nosema-ceranae-the-inside-story)

nature of that combination remains uncertain.[11, 12] For purposes of this guidance, incidents of CCD are not included in the term "bee kill incident," although inspectors should be aware of its existence.

Bee Diseases and Other Factors

Bee kill events may result from a number of factors besides pesticides. These can include sudden changes in temperature (*e.g.*, brood chill), opportunistic predators (*e.g.*, European hornets, yellow jackets, small hive beetle, skunks, *etc.*) and disease.[13] In some cases, losses associated with disease can be misinterpreted as pesticide exposure related losses and it can be difficult for inspectors to distinguish whether disease is a factor. Even experienced beekeepers may not notice that a particular disease is a factor, especially if their initial observations focused on the dead/dying bees outside of the colony entrance. (It is common bee behavior to remove all dead bees from inside the hive, regardless of what caused their death, and discard them outside the hive's entrance.) It is not possible to provide an in-depth account of all the possible bacterial,[14] fungal and viral diseases to which bees may be subject to in this guidance. However, inspectors must be aware that diseased colonies can result in the loss of large numbers of both adult bees and their developing brood. Where disease is suspected, samples of colony matrices can be collected for diagnosis.

Pesticide Exposure

Bee kill incidents may also be caused by exposure to pesticides. Colony exposure may occur through drift of pesticides from aerial or ground applications immediately adjacent to where colonies are located and/or to areas where bees may be foraging for food and/or water. In some cases, exposure may occur through a direct overspray event. While direct exposure to a pesticide through drift can result in acute mortality and the loss of large numbers of forage bees in the field or at the entrance to colonies, pesticides can also be brought back to the colony by forage bees either through contaminated nectar and/or pollen, or through residues on the surface of the bee (*e.g.*, dusts).

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[11] VanEngelsdorp, D., J. D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B. K, Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. Chen, R. Underwood, D. R. Tarpy, J. S. Pettis. 2009. Colony Collapse Disorder: A Descriptive Study. PLoSONE 4(8): e6481. Doi:10.1371/journal.pone.0006481http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0006481.

[12] For more information on CCD, refer to the USDA Agricultural Research Service website: http://www.ars.usda.gov/News/docs.htm?docid=15572.

[13] One such bacterial disease, American foulbrood, is caused by the spore-forming bacterium Paenibacillus larvae and is considered by USDA to be the most serious infectious disease of honey bees. For more information on the detection and treatment of this disease in bees, refer to http://naldc.nal.usda.gov/download/28123/PDF.

[14] There are many publications that provide additional information on the diagnosis of honey bee diseases. For example, the USDA Agriculture Handbook Number 690 (Diagnosis of Honey Bee Diseases) is available online: http://www.ars.usda.gov/is/np/honeybeediseases/honeybeediseases.pdf. Also, the Food and Agriculture Organization of the United Nations has published an Agriculture and Food Engineering Technical Report 4 online entitled: Honey bee diseases and pests: a practical guide; this report can be accessed through: http://www.fsnnetwork.org/resource-library/ag-nutrition-health-linkages/honey-bee-diseases-and-pests-practical-guide. Reference to these documents is not intended as an endorsement of the treatment methods discussed in the documents; rather the documents are cited with respect to the methods used in diagnosing bee colony diseases.

Effects of Pesticide Exposure

Once inside the colony, pesticide residues may result in effects to hive bees (*e.g.*, nurse bees, the queen) and/or developing larvae and pupae (collectively referred to as "brood"). These effects may not be expressed as the sudden loss of large numbers of bees in close proximity to the outside of the colony but can also result in the diminished overall strength of the colony as reflected by the size of the cluster of adult bees on the comb or in terms of the general pattern of brood, *i.e.*, spotty as opposed to uniform brood areas. While the majority of bee kill incident reports are associated with an acute mortality event as indicated by large piles of dead adult bees at the entrances to colonies, bee/hive health problems may be reported and may require inspection outside the typical bee kill inspection process by individuals other than the pesticide state lead agency.

Challenges for Inspecting Bee Kill Incidents

There is a broad range in the number of bee hives maintained by beekeepers. The American Beekeeping Federation has defined the following categories: small scale (<25 colonies), sideliners (25 – 300 colonies) and commercial (>300 colonies).[15] For large-scale commercial beekeepers which provide pollination services and/or honey production, the number of colonies may extend into the tens of thousands of colonies and these colonies can be distributed over considerable distances. While small scale beekeepers may have colonies in close proximity to their residences, many of the sideliners and commercial beekeepers are not in close proximity to their colonies and may not routinely check the status of each colony. Therefore, the ability of a beekeeper to immediately detect a bee kill incident depends on the frequency at which they monitor their colonies and it is possible that they discover the loss at a time that is well after the bee kill incident occurred. The inspector should take this elapsed time into account when making observations or collecting samples and pesticide use information at the bee kill site. Also, given that many bee yards (apiaries)[16] where colonies may be positioned are located away from where the beekeeper resides, the events surrounding the loss may not be readily apparent. Ideally, a bee kill incident should be reported as soon as it occurs. The reality may be that such incidents are reported several days or longer after the initiating event occurred. The timeliness of such reports can affect the ability of either the beekeeper or an inspector to determine the cause.

Bee kill incidents suspected or known to be related to pesticide exposures are reported through a variety of mechanisms. When tips and complaints[17] alleging pesticide related bee kill incidents are quickly submitted directly to state lead agency or tribal representatives by members of the public such as beekeepers, states/tribes can respond in a timely manner to investigate the incident. However, incident reports are often made to other regulatory agencies such as EPA's Office of Pesticide Programs (OPP) (*e.g.*, beekill@epa.gov), through data collection centers (*e.g.*, National Pesticide Information

[15] American Beekeeping Federation. 2012. http://abfnet.org/displaycommon.cfm?an=1&subarticlenbr=18 Accessed 12/09/2012.

[16] Many states require, while some states recommend, the registration of apiaries. Although not available in all states, these registries provide the location of bee colonies and depending on the nature of the registry, can afford some advanced notice if aerial pesticide applications are planned for areas adjacent to the apiary. However, bees conducting pollination services may be exempt from registration.

[17] Any tip or complaint may be submitted to EPA directly at http://www.epa.gov/tips/.

Center; http://pi.ace.orst.edu/erep/), to pesticide registrants (typically listed as contacts on pesticide labels) and to the press. Incidents reported to these other entities may not get reported to the appropriate state lead agency for proper and timely inspection follow-up.

Pesticides can affect both target and non-target organisms in different ways depending on the mode of action of the compound. Depending on the mode of action and level/duration of exposure to a pesticide, the effects exhibited by bees can differ and can extend over a broad range of signs including behavioral changes [walking instead of flying, inactivity, excessive grooming, extended tongue (proboscis), jerky movements] as well as death. Bees that appear to be writhing and or walking on the ground should be noted by the inspector. As with most non-target incidents, bee kills typically represent acute mortality events. The inspector should be aware that the bee kill may have occurred as a result of either an acute or a chronic exposure to a pesticide or pesticides.

Bees can die on their way to and from the colony, at the entrance to the colony and within the colony. When inspecting a bee kill incident, the inspector should attempt to estimate the number of dead bees in these different areas. Minimally, bees at the base of the outside hive entrance should be examined. Acute mortality events from pesticide exposure are typically expressed as a pile of dead bees at this location. Field force knockdown[18] can also be observed within 25 feet of the colony for workers that are unable to return. Beyond this, the area may be obscured by ground cover which will block observation. In reporting the nature (condition) of the dead and disabled bees, the inspector should attempt to discern whether different aged (larvae, pupae, young adult) bees or castes (workers, drones, and queen) appear affected[19]. Age and caste may be relevant to determining if the bee death is a result of pesticides or other causes. For example, late season death of drones alone may be attributed to the nature of the hive where drones are expelled from the colony[20] as it prepares for overwintering. Larvae may be affected by pollen collected and fed to the developing bee. In order to assess overall hive health and understand whether factors other than pesticide exposure may be affecting the hive, it may be necessary to examine the interior of the colony. If the hive is opened, it can be examined for the extent of dead/disabled bees on the bottom board of the colony, the extent to which individual frames are covered by bees (cluster size), whether the brood pattern is relatively consistent as opposed to spotty[21], and in some cases identify disease and parasite damage. For example, a large infestation of wax moths would indicate that the colony was lost at least one month before.

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[18] Field force knockdown typically refers to the rapid loss of worker bees that are actively engaged in foraging. These losses may result in large losses of bees in the treated field as opposed to at the entrance to the colony.

[19] Younger bees generally work inside the colony. As [worker] bees age they will start to forage and continue foraging until they die (Winston, M.L. 1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, MA.)

[20] Late season loss of drones occurs very late in the fall; drones can be displaced during a “dearth” or lack of nectar flow, even in mid-summer. Drones can be expelled from the hive due to season and/or stress (*Ibid* Winston 1987); (Sammataro, D. and A Avitabile. 2011. The Beekeeper's Handbook. Fourth Edition. Cornell University Press, Ithaca, NY).

[21] Spotty brood can be caused by diseases, such as Chalk Brood, by an old failing queen, or a number of other problems and should not be considered diagnostic in and of itself; it is a sign that something is wrong. (Sammataro, D. and A. Avitabile. 2011. The Beekeeper’s Handbook. Fourth Edition. Cornell University Press, Ithaca, NY.).

While the inspector may not have sufficient knowledge of bees to approach and disassemble a colony and/or to discriminate this level of detail, the beekeeper should be able to assist in this effort. The inspector may be able to observe or document signs of decay which depend to some extent on whether the dead bees are dry or wet. In general, these signs are as follows:

- loss of the fine hairs that typically cover the bodies of honey bee;
- loss of wings;
- putrid odor;
- darkening color;
- insect body becomes more amorphous (difficult to distinguish individual bees, body is desiccated and/or dismembered).

Within 3 days of the bee kill incident, the loss of hairs and the stench would likely be apparent. Within 14 days, wing/leg loss would be observed and by 30 days, the inspector will likely observe bee body parts with few to no intact bodies.

Because bees can forage over considerable distances, their survival can be impacted by a broad range of environments (e.g., agricultural, nonagricultural, residential, forests) that may be within their flight range. While bees will forage to meet the nutritional/energy needs of the colony and typically select forage that represents a preferred source of both pollen/nectar, they may also forage on less preferred sources of nutrition/water based on availability. While it is not possible to state a specific distance that must be considered in terms of the location of affected colonies to potential sources of pesticide exposure, there are practical limitations to how much effort can be expended by an inspector. In some cases, the beekeeper may be able to provide information on the suspected pesticide use. However, in the absence of such information, the inspection may have to be limited to a specific perimeter (See Footnote 15, page 6) that should be noted in the report. A general observation of the area surrounding the apiary, whether orchard, field crop, vineyard, or other possible pesticide use site should be considered along with time of year to determine a likely source of pesticide exposure. Based on the inspector's knowledge of pesticide application practices, crops and application timing, it may be possible to determine a likely source of pesticide exposure. In addition to crops and practices, inspectors will need to be observant for blooming crops, wildflowers and other plants in the area of the affected apiary within the selected inspection perimeter.

It may be difficult to determine if a bee kill is related to treatment of adjacent fields because there is no easy way to know where bees have been foraging. Dead bees near a treated field would be important to investigate. Initially, the inspector should try to determine where bee deaths are occurring and then determine where pesticide use has recently occurred or is occurring in the general area. To the extent possible, it is important to consider the timing of applications relative to the mortality events. If there are other apiaries in the area, the inspector should attempt to contact the beekeepers to determine if others in the area have experienced bee kills. Apiary locations are typically well hidden to limit the chance of vandalism. If other apiaries are similarly affected, it may provide additional insight into the location of pesticide exposure. Priority should be given to the apiary experiencing the bee kill, if there are several apiaries in the area. Note that bees forage based on the identification of a nectar

source. All apiaries in an area may not identify the same source, so the pattern of damage in a geographical area can be inconsistent. When trying to locate a source of pesticide exposure, the inspector should work his/her way from the affected hive(s) outward.

ATTACHMENT IV

Photographs

Figure 1. Honey bee colony opened for inspection showing large numbers of bees covering the tops of most of the frames in the bottom box. Note that the colony to the right has considerably fewer bees on top of the visible frames (indication of reduced colony strength). A smoker unit is to the right of the upright colony and is used to clear bees from the colony and make them less likely to sting. Additional commercial colonies can be seen in the background as pollinator services are provided to almond orchards.

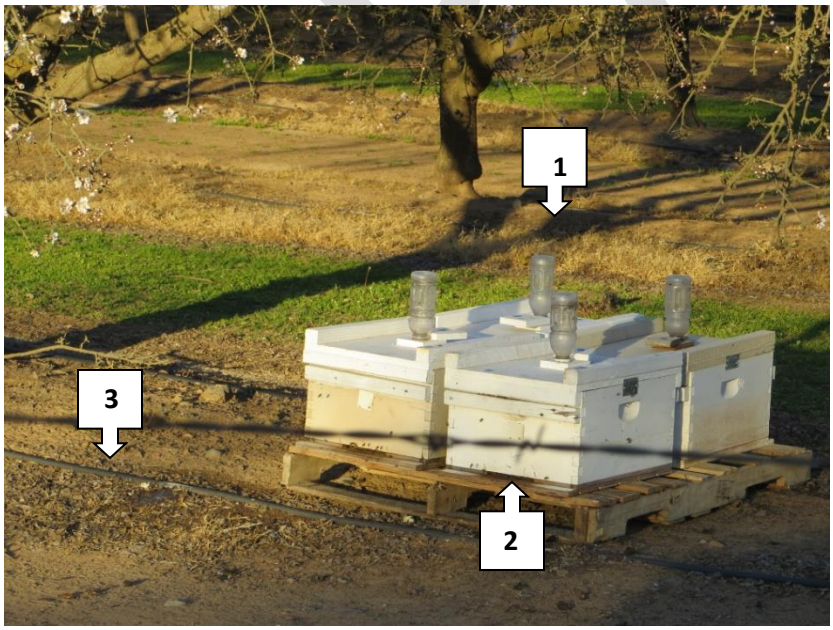

Figure 2. Commercial honey bee colonies on pallet in California almond orchard.

1) Plastic bottles atop each of the colonies used to supply water to the colonies.

2) The narrow entrances of the colonies are the centermost spaces at the base of each colony.

3) Irrigation line could serve as a potential source of exposure to bees if pesticides are present in the water (chemigation) or on the ground.

Photos by: Anita Pease, Office of Pesticide Programs, EPA

ATTACHMENT IV

Photographs

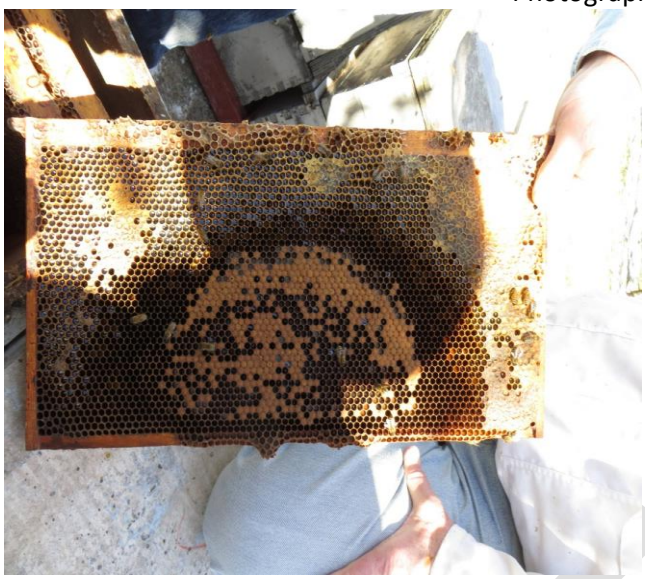

Figure 3. Frame from honey bee colony showing unhealthy spotty brood pattern. Frame also exhibits the concentric pattern of interior brood cells surrounded by pollen cells surrounded by honey cells.

Figure 4. Frame from honey bee colony depicting central cluster of bees; relatively uniform and healthy sealed brood (pupal) cells are to the right of the cluster of bees.

Photos by: Anita Pease, Office of Pesticide Programs, EPA

Figure 5. Honey bee colonies opened for inspection to reveal interior frames. This is an example of a dead colony. Further investigation would be needed to determine whether pesticide exposure could be a cause.

1) A reservoir used for feeding sugar solution (carbohydrate source).

2) Pollen cakes used as a source of protein for bees when natural sources of pollen are limited.

3) Note that the irrigation canal directly behind the colonies could be considered a potential source of exposure to bees if pesticides are present in drinking water.

Figure 6. Close-up view of young worker bee ("fuzzy" appearance suggests that this is a recently emerged bee). A varroa mite (*Varroa destructor*) can be seen toward the base of the wing (shiny brown object).

Photos by: Anita Pease, Office of Pesticide Programs, EPA

Attachment V
Example On-Site Hive Inspection Checklist

Inspector:	**Date of Inspection:**
Time:	**Credentials Presented?** YES NO
	Notice of Inspection Presented? YES NO
Responsible Individual: (*e.g.*, beekeeper)	**Address/Phone/Email:**
Beekeeper/Owner: (if not in attendance, include contact information)	
Inspection Participants: (identify all other participants including SLA inspectors, state apiarists, *etc.*)	
Inspection Location: (description or address, or attach map/diagram showing nearby features relative to bee kill site) Apiary Registration Number: (if applicable)_____ Map/Diagram of Bee Kill Incident Attached? YES NO	
Bee Kill Incident Beekeeper or owner's description of incident: Symptoms or adverse effects noted:_____ Estimated # of bees killed/adversely affected: _____ Number of hives affected:_____ Proportion of bees affected per hive:_____ Location of incident:_____ Written statement collected from beekeeper/owner? YES NO	

Attachment V
Example On-Site Hive Inspection Checklist

Health of Hive/Colony/Apiary

Describe strength of colony:_____

Are there any signs of disease or parasites? YES NO

If yes, describe:_____

Has there been active treatment of the hive(s)? YES NO

If yes, note signs: _____

Records of active treatment collected? YES NO

Were any management techniques implemented in prior 3 weeks? YES NO

If yes, describe:_____

Has the beekeeper done any feeding/watering of the hives? YES NO

If yes, describe:_____

Was the hive/apiary involved in research? YES NO

If yes, describe:_____

Commercial colonies

Where were colonies before incident?_____

When did colonies arrive at site?_____

Was honey removed prior to colonies' arrival? YES NO

If yes, When:_____

Were colonies inspected prior to their arrival? YES NO

If yes, When:_____

Was the colony inspected by independent apiarist prior to moving? YES NO

If yes, was a written inspection report available and collected? YES NO

Attachment V
Example On-Site Hive Inspection Checklist

Pesticide Applications to the Hive/Colony/Apiary

Were pesticides applied to the hives in the last year? YES NO

If yes, who was the applicator?_____

Record/collect the following for each product applied (attach additional pages as needed):

Product Name: _____

EPA Reg. No.:_____

Active Ingredient:_____

EPA Est. No.:_____

Distributor Name/Address:_____

Date(s) of Application:_____

Applicator (if not beekeeper):_____

If applied in last 3 months, label collected? YES NO

Physical sample collected? YES NO

Documentary samples (labels, records of sale/receipt) collected? YES NO

Inspection of and Sample Collection from the Hive

Are any dead bees observed? YES NO

If yes, note location:_____

Photograph hive and bees, including dead bees YES NO

Physical Samples Collected (as applicable):

	Collected?	**Collected By**	**Location of Sample Collection**
Dead and/or abnormally behaving bees Sample No_____	☐		

Attachment V
Example On-Site Hive Inspection Checklist

Honey/honeycomb Sample No._____	☐		
Swab samples from hive exterior Sample No. _____	☐		
Vegetation samples around hive or incident location Sample No._____	☐		
Food, water, or other medium provided to bees by beekeeper or other individual Sample No._____	☐		
List or attach other samples collected (as needed):			
Receipt for Samples Provided?	YES	NO	
Time at end of Inspection:			