Worker Reentry into Pesticide-Treated Crops. I. Procedure for the Determination of Dislodgable Pesticide Residues on Foliage[1]

Y. Iwata
Department of Entomology
University of California
Riverside, Calif. 92521

J. B. Knaak
California Department of Food and Agriculture
Sacramento, Calif. 95814

R. C. Spear
School of Public Health
University of California
Berkeley, Calif. 94720

R. J. Foster
Department of Agricultural Chemistry
Washington State University
Pullman, Wash. 99163

Agricultural workers have on occasions become ill as a result of entering and working in a field some time after a pesticide application had been made to a crop plant. This problem is associated with exposure to cholinesterase-inhibiting pesticide residues. Since reports involving illness have been largely limited to tree fruits, grapes, tobacco, and cotton where continuous and extensive contact with treated foliage and other plant surfaces is involved, foliage is suspected of being the prime source of toxicants to workers. Dislodgable pesticide residues residing on plant surfaces or sorbed to particulate matter on foliage can be transferred to worker skin and clothing and result in dermal exposure of workers to toxic materials. Although the relationship between foliar residues and an adverse biochemical response of workers has not been clearly established, the identity, levels, and behavior of the dislodgable foliar residues need to be known as a first step toward providing for worker safety. This "reentry problem" has received considerable attention from a number of research groups. For a greater in-depth discussion of aspects of the reentry problem, the reader is referred to MILBY (1974), SPEAR *et al.* (1975), GUNTHER *et al.* (1977), KILGORE (1977), and references therein.

With the diverse research groups involved in measuring and reporting levels of dislodgable residues on foliage, it is desirable that a common methodology be used so that residue data reported by various groups are comparable. This report consolidates in one place the main points of the dislodgable foliar residue procedure which was initially developed by Gunther and co-workers (GUNTHER *et al.*, 1973 and 1974; WESTLAKE *et al.* 1973). The goal here is not to present an inflexible sequence of operations that must be followed but to present a procedure that diverse groups will find sufficiently reasonable and easy to adopt or approximate.

Any technique to evaluate total dislodgable residues must accommodate statistical size and appropriate field representation requirements, be simple, yet be a standardized technique which

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will be reproducible, and be adaptable to a variety of pesticides. The technique must not be so tedious that the operator becomes bored and careless during the collection of large numbers of samples resulting from replicate sampling in replicated field plots or a large number of groves. Foliage surface areas and often weights of the samples are frequently desired. Collection of leaf disc samples of fixed diameter was determined to be a reliable procedure. An appropriate leaf punch should have a strongly concaved cutting surface to avoid disturbance of the foliar surface. The excised disc should fall into the sample container without operator handling and a stroke-activated counter should record the number of disc samples collected. Field experience has demonstrated that the cutting edge will need to be cleansed after each batch sample with a tissue moistened with water or acetone to remove plant juices, to maintain easy operation of the punch, and to prevent cross-contamination between samples. Further, the number of leaf discs collected should be confirmed in the laboratory after the dislodgable residue removal step is completed.

A schematic diagram of a leaf punch sampler which has been found satisfactory is shown in Figure 1. This particular sampler is produced by the Birkestrand Company (2563 Loma Avenue, South El Monte, CA, 91733). The collection of a one-inch (2.54 cm) diameter disc is recommended; the exact diameter selected may be somewhat larger or smaller to suit the needs of the researcher. For example, narrow leaves such as those from peach trees may require collection of smaller diameter leaf discs. An 8-oz (240 ml) glass jar is suitable for the collection jar attached to the leaf punch sampler. A jar this large reduces the likelihood of clumping of the leaf discs that could cause incomplete recovery during the dislodgable residue removal step. An adequate sample is 40 or more leaf discs per sample; the total surface area for a sample of 40 leaf discs of 2.54-cm diameter is 405 cm^2 (two sides). Use of jars engraved with the tare weight will allow facile determination of the foliar weight by weighing the sample jar after collection of the leaf discs.

The leaves should be free of excess moisture at the time of sampling; moisture resulting from a spray application, rain, overhead sprinkler irrigation, or morning dew should be allowed to evaporate before sampling is undertaken. For citrus, the tender developing or newly-mature, first-cycle leaves should not be used and only the fully-mature, second-cycle leaves should be sampled. Residues on young leaves may be subject to growth dilution and since surface characteristics of young leaves differ from those of older leaves, residue behavior may also differ. Leaf discs should be taken near the center of the leaf. For non-citrus crops, samples are taken from healthy, averaged-sized leaves. Leaf discs are taken where the leaf appears to represent the average thickness. A minimum of two replicate samples should always be taken from a given plot or area of interest.

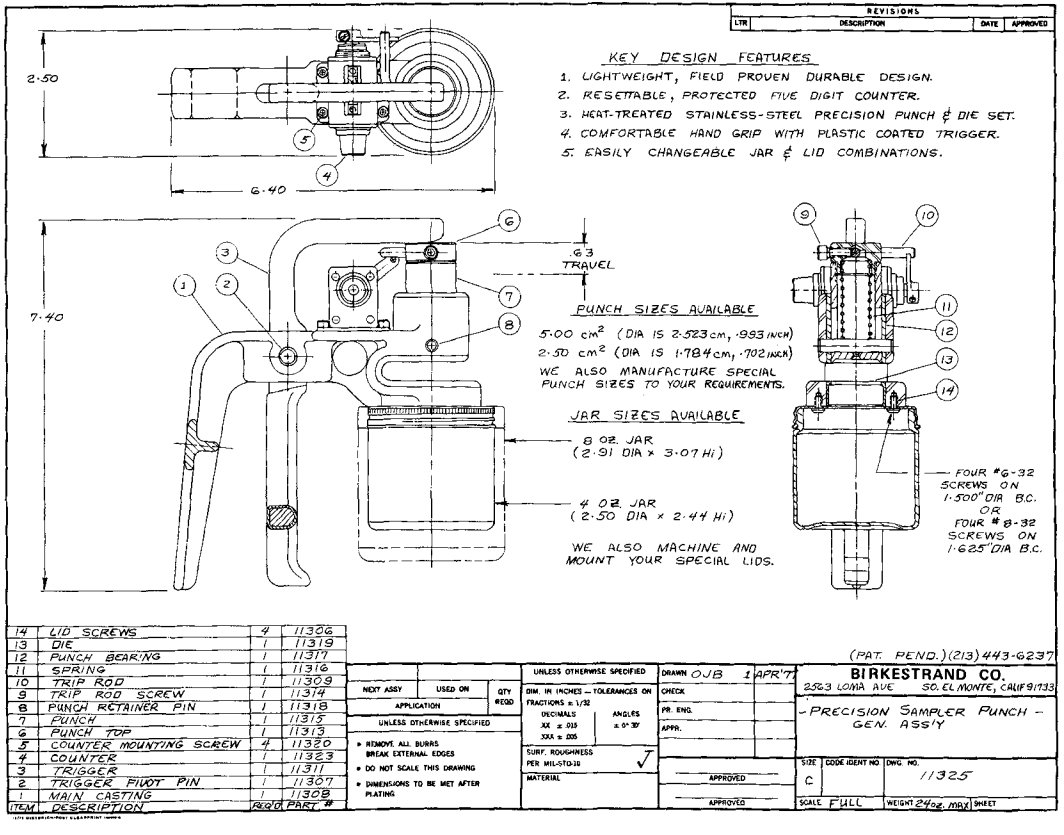

Figure 1. Schematic diagram of a leaf punch sampler.

For citrus trees, the portion of the tree about four to six feet above ground is sampled. One sampling procedure for obtaining 40 discs is to punch at 45° intervals around each of eight trees, with five discs per tree as follows to afford five discs per sampling position:

Tree 1	0°	45°	90°	135°	180°
Tree 2	45°	90°	135°	180°	225°
Tree 3	90°	135°	180°	225°	270°
Tree 4	135°	180°	225°	270°	315°
Tree 5	130°	225°	270°	315°	0°
Tree 6	225°	270°	315°	0°	45°
Tree 7	270°	315°	0°	45°	90°
Tree 8	315°	0°	45°	90°	135°

Another method which simplifies the sample collection procedure and increases the sample size (total area) but decreases the number of trees sampled is to collect a total of 48 leaf discs from eight points equally spaced around each of six trees. Any sampling procedure can be substituted provided that the entire circumference of the tree is represented.

Many crops will have their unique sampling problems. For small, low crops, samples should be taken from each of the four 90° positions around the circumference of the plant; the discs, however, need not be taken from a single plant. Leaf discs may not be feasible due to the size or irregular shape of the leaves. Entire leaves may then need to be sampled and known areas cut out from the leaves or if the area cannot be determined (parsley, for example), the dislodgable residue may have to be expressed on a weight basis. The leaves sampled should represent the foliage that workers are most likely to contact, such as the wrapper leaves of lettuce and cauliflower. With tall, upright crops such as cotton, staked tomato, grape, and sweet corn, leaf samples should be taken from the lower, middle, and upper portions of the plant canopy. Foliage from tree crops other than citrus are sampled at the highest level accessible by a sampler standing on the ground. Ladders are to be avoided if possible. If an aircraft application has been made such that the tops of trees are likely to have higher foliar residues than the lower portions and workers are likely to contact this foliage, then provisions to sample this portion of the tree should be made.

Depending on the user's need, samples should be collected to fairly represent either the entire plant population or a small, defined segment of the field. In the first case, knowledge of the application procedures is vital. In uniform fields, a purely random array of plants would be adequate; where nonuniformities in the application procedure are either known or suspected, then a stratified random sampling strategy should be established which

will either proportionately represent the nonuniform zones or completely exclude them. For the purposes of residue dissipation studies, a relatively closely distributed group of plants interior to the field may be selected for repeated samples over time.

When conducting a residue dissipation study, the recommended procedure is to establish three replicate plots for a specific treatment and to obtain duplicate leaf-disc samples from each plot at each sampling interval. The intervals selected will depend on the commodity and pesticide applied.

For spot-checking residue levels in a large number of fields, a minimum of two replicate samples should be taken from the area of interest for immediate residue determination. If the situation warrants it, additional samples may be collected and stored pending the outcome of the analyses of the two survey samples. Specific trees and fields should be tagged if multiple samplings at different time intervals are planned. The highest value obtained from the two or more samples analyzed should be used to characterize the maximum hazard existent in the area sampled.

Collected samples in the field and in transit to the laboratory should be kept cool. To avoid unnecessary complications, storage of leaf discs in any manner should be avoided by provisions for immediate processing. If discs must be stored, preliminary tests should be made to determine the extent of adverse storage effects, if any.

For removing dislodgable residues, use of an organic solvent is best avoided as it can carry external residues into the leaf tissues or, perhaps more seriously, extract penetrated residues. The recommended procedure given below is to mechanically shake the leaf disc sample with water containing Sur-Ten (70% dioctylsulfosuccinate sodium salt) or an equally effective wetting agent. The residues are then extracted from the aqueous wash into an organic solvent as follows:

1. Add 100 ml of water and 4 drops of a 1:50 dilution of Sur-Ten wetting agent to an 8-oz widemouthed jar containing 40 leaf discs.
2. Cap the jar and shake on a reciprocating shaker at 200 strokes per min for 20 min.
3. Decant the liquid into a 500-ml separatory funnel (or temporary holding bottle) retaining the leaf discs in the jar and paying particular attention to transferring the associated leaf dust to the separatory funnel.
4. Repeat the above procedure two more times, adding the liquid from each to the first.
5. Add 50 ml of CH_2Cl_2 and shake for 1 min.
6. Drain the lower layer through a funnel containing about 10 g of Na_2SO_4 into a sample storage bottle.

7. Repeat with 50 ml of CH_2Cl_2 and 1 min shaking.
8. Wash the funnel and Na_2SO_4 with about 10 ml of CH_2Cl_2.
9. Remove the CH_2Cl_2.
10. Dissolve the residue in acetone or hexane for glc analysis.

The partitioning process should be checked for each compound sought by fortifying a simulated leaf wash mixture or a leaf wash of a control sample with appropriate levels of each sought compound and verifying that satisfactory amounts are transferred into the organic solvent. This is particularly necessary when the sought compound has appreciable water solubility. The above procedure is presented as an example which should be generally applicable.

Final results for the dislodgable residues should be expressed in units of µg of compound per cm^2 of leaf area. The use of "ppm" should be reserved for expressing residues present within the leaf or on/in foliar dust. The dislodgable foliar residues are sorbed to the particulate matter resident on the leaves. The dust is the vehicle by which toxicants are transferred from foliage to workers and large amounts of foliar dust may represent greater worker hazard. A procedure for quantitating the foliar dust has been given by POPENDORF and LEFFINGWELL (1977).

Application parameters and pertinent climatic data should be recorded when multiple sampling programs are undertaken to determine if any correlation can be made with the residue data.

Considerable quantities of pesticides reach the soil surface through spray runoff and drift, and the residues on the mobile dust may be important under some circumstances in contributing to worker hazard. A procedure for the determination of pesticide residues on the soil surface is described by SPENCER et al (1977).

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