PART B - CHAPTER 3

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DISLODGEABLE FOLIAR RESIDUE DISSIPATION: AGRICULTURAL GUIDELINE 875.2100

3.1 INTRODUCTION

This Guideline provides a description of the techniques and sampling strategies commonly used to characterize dislodgeable foliar residue (DFR) dissipation. Such dissipation data are used in conjunction with concurrent human exposure data to establish chemical transfer coefficients, to determine restricted-entry intervals, and to evaluate risks associated with postapplication exposure to pesticides. DFRs represent chemical residues on the surfaces of treated foliage that are available for transfer to exposed populations (e.g., reentry workers) during contact with those treated leaf surfaces. That is, DFRs are the amount of chemical residues deposited onto the leaf surface that have not been absorbed into the leaf or dissipated from the surface, and that can be dislodged by shaking leaf samples in a detergent solution. (See Gunther et al., 1973.)

3.2 SAMPLE COLLECTION

3.2.1 Test Substance

As stated at 40 CFR 158.390, the test substance to be used for inhalation exposure measurements must be a typical end-use product. Where metabolites, breakdown components, or contaminants of pesticide end-use products pose a potential toxicological concern, investigators may need to consider sampling for them on a case-by-case basis.

3.2.2 Timing of Application

Sample collection should be conducted during the intended use season or under climatic conditions that are essentially identical to those encountered during the intended use season. Weather forecasts should be studied to avoid initiating the testing immediately (e.g., within 24 hours) before a precipitation event. For further information on climatological considerations, see Part B, Chapter 2 - Study Design.

3.2.3 Pesticide Application Rate and Frequency

Generally, the end-use product chosen for the study should be applied at the maximum rate specified on the label. In addition to applying the product at the maximum label rate, it is suggested that the

product be applied using a lower application rate, if possible. For example, typical rates are often used in cancer assessments (U.S. EPA, 1997). Monitoring at more than one rate will also provide additional information about the relationship between the application and deposition rates. Also, testing at a lower rate may prove to be beneficial in the event that the data from use of the product at the maximum application rate results in an unacceptable risk.

Where multiple applications are recommended, the minimum time interval between applications should be used. Also, testing at a longer time interval between applications may prove to be beneficial if unacceptable risks were to result. Registrants should note, however, that if the maximum rates or minimum application intervals are not monitored, subsequent label changes may be necessary. Also, the potential accumulation of residues from multiple applications should be considered. The application method and equipment typical for the selected test substance should be used.

3.2.4 Sampling Parameters

The following paragraphs provide a description of: where geographically sampling should occur (i.e., site selection); how long the dissipation must be characterized (i.e., the sampling period); the times within the sampling period that samples should be taken (i.e., sampling intervals); and the number of samples that should be taken at each sampling interval along with a description of where at the sampling location to sample.

3.2.4.1 *Number of Geographic Locations*

In general, DFR samples are typically collected from at least three geographically distinct locations per formulation type. This is usually necessary to ensure that varying climatic conditions, crops, and pest types are represented. (See Chapter 2, Study Design, for more guidance.)

3.2.4.2 *Sampling Period*

Data should be collected in a manner that characterizes the dissipation mechanisms for the compound (e.g., three half-lives). Further, the sampling period should be reflective of the exposure conditions and toxicological endpoint of concern (i.e., acute or chronic). Typically, DFR dissipation rates are characterized for at least 35 days postapplication for agricultural sites unless the compound has been found to fully dissipate in less than this time period. EPA has observed that this sampling period is usually adequate for characterizing pesticide dissipation under most use conditions; for most pesticides, significant dissipation

occurs within the first week of application. Please note, however, that for more persistent pesticides a longer sampling period may need to be used.

3.2.4.3 ***Sampling Intervals***

Generally, the length of time between sampling should be relatively short in the beginning and should lengthen as the study progresses. EPA recommends that samples be collected prior to application on the day of application; on the application day at various hourly intervals after application (e.g., 4 hours or 12 hours after application may be appropriate); and on various days postapplication. For example, sampling at 1, 2, 4, 7, 10, 14, 21, 28, and 35 days after application may be appropriate. Please note that for certain pesticides (e.g., one that degrades quickly) shorter sampling intervals may suffice. However, sampling should continue for 35 days, but all of the samples may not need to be analyzed if the pesticide has fully dissipated (i.e., nondetects for two sampling intervals). On the other hand, sampling may need to continue beyond 35 days for more persistent pesticides. Special consideration should also be given to pesticides that exhibit biphasic dissipation kinetics.

If typical chemical use patterns involve a sequential series of treatments (i.e., multiple applications), samples in addition to the ones indicated above should be collected. Generally for multiapplication scenarios, the Agency recommends that samples be collected prior to and after each application on the day of application. Additionally, after all but the final applications (see above), samples should be collected at least every 7 days after each application during the intervals between application events (e.g., if multiple applications occur on a 14 day interval, then samples should be collected prior to and after each application and 7 days after each application). However, modifications to this sampling scheme may be proposed to the Agency if the market and use pattern will accept that reentry is prohibited between applications, or if the registrant is willing to accept the results and restrictions imposed by the alternative sampling scheme.

The proposed sampling intervals should be presented to EPA for review in the study protocol prior to initiation of the study to ensure that they are agreeable to the Agency.

3.2.4.4 ***Number of Samples and Sampling Positions***

The Agency recommends that at least three samples be collected at each sampling interval. Each of the replicate samples should be taken from different areas within the treated location. Sampling should occur in the worker contact zone (i.e., in those areas where workers will be conducting their activities). Several approaches have been utilized by investigators to determine where to sample within treated areas. These include, but are not limited to: nondirected sampling; the Iwata approach for tree crops (Iwata et al., 1977);

and the planned approach for row crops. Control plots should also be established. Sufficient control samples should be collected to ensure that the same bulk sample can be used as a negative control matrix throughout all sample analyses. Additionally, samples from the control plots should be collected at each interval for assessment of the field sample collection and storage procedures.

In nondirected sampling, field technicians enter a treated area and sample at their own discretion. The Iwata approach provides guidance for collecting DFR samples from tree crops. Samples are collected at 45 degree intervals around the circumference of each sampled tree and at varying heights in the tree (see Figure B3-1). In the planned approach, investigators develop a scheme that predetermines sample collection locations (see Figure B3-2). EPA recommends that a planned approach be used for DFR sample collections for typical field crops (e.g., row crops such as strawberries or tomatoes) and that the Iwata approach be used for tree crops.

3.2.5 Technique Validation

The concept of dislodgeable foliar residue sampling is based on the assumption that available residues are removed from the surface of treated foliage by washing (i.e., dislodging) with an aqueous surfactant solution. However, data are not available to quantify the actual rate of transfer from the treated surface into solution (i.e., no mass balance data are available). As a result, the only validation that is routinely required by the Agency is to determine the efficiency of extraction of the chemical residue(s) of concern from the dislodging solution and the stability of those residues in the solution over time if sample storage is required prior to analysis. No further validation data are required until a technique becomes available to accurately quantify the mass balance (i.e., efficiency of the dislodging procedure).

3.2.6 Sampling Techniques

There are two types of approaches for monitoring dislodgeable foliar residue levels (i.e., leaf punch and whole leaf sampling). The equipment required for each approach is described below. For all studies, a leaf punch apparatus should be used unless the nature of the crop (e.g., small leaves) precludes its use. Additionally, aqueous dislodging solutions should be used.

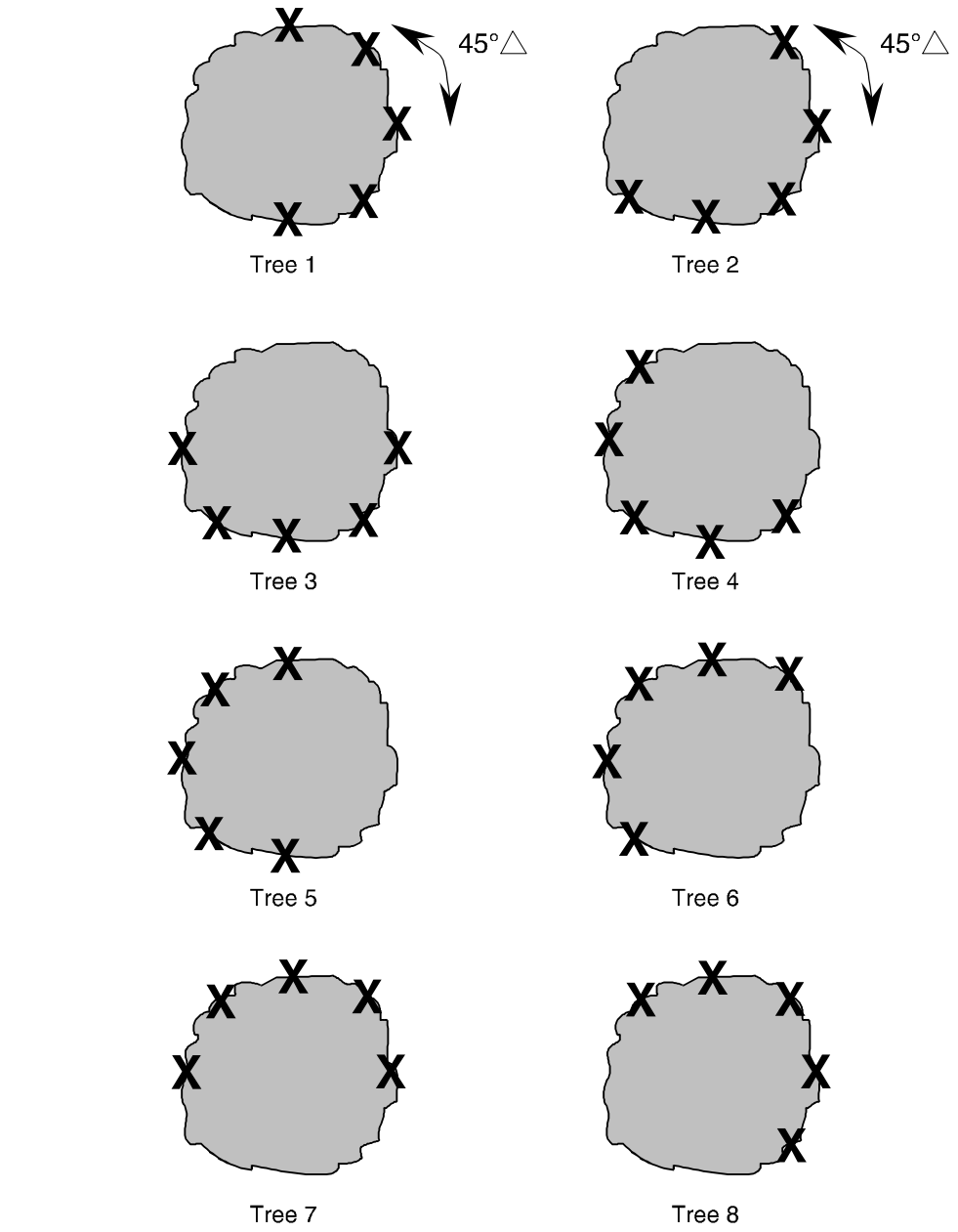

Figure B3-1. IWATA Approach for Tree Crop Sampling

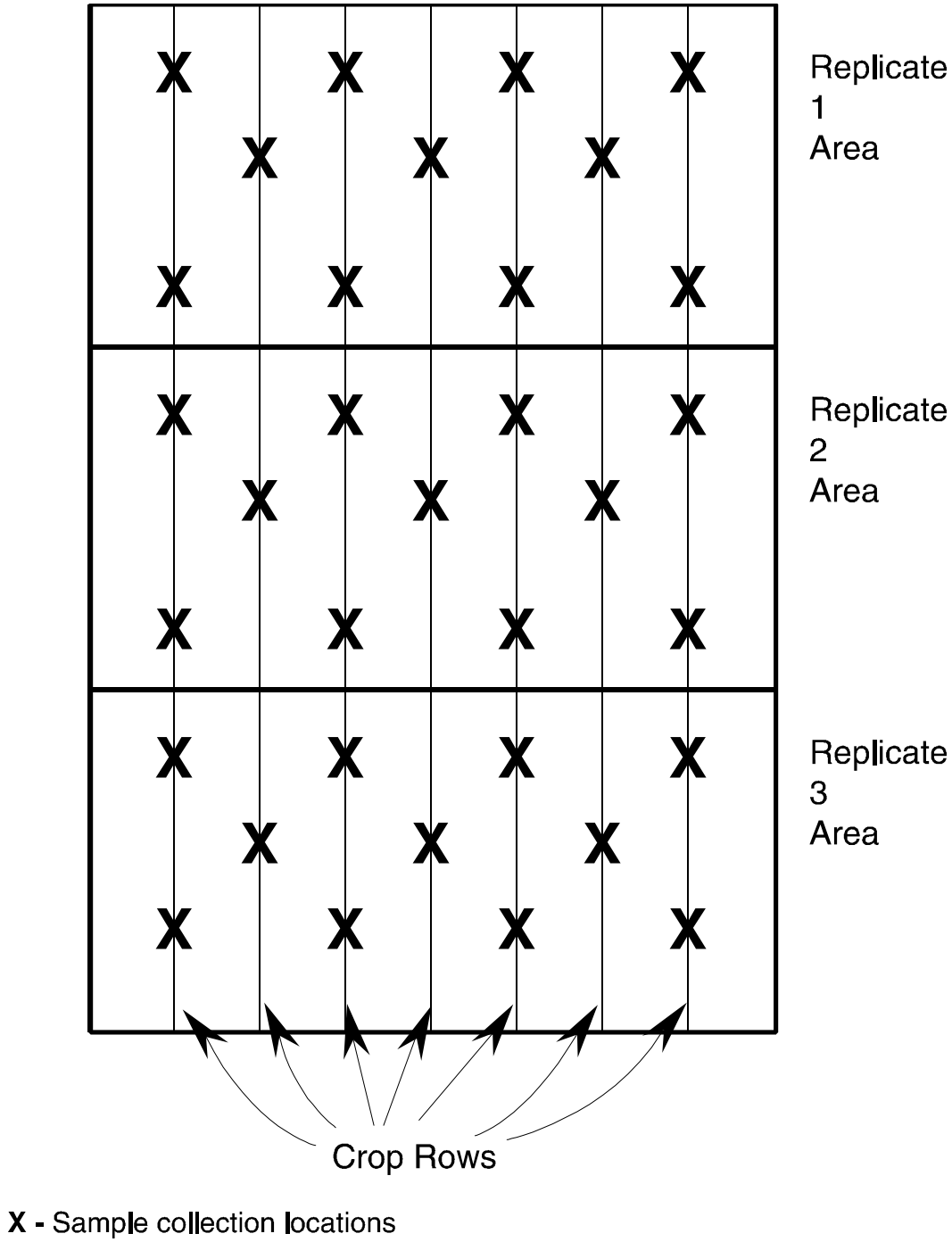

Figure B3-2. Planned Approach for Field Crop Sampling

3.2.6.1 ***Leaf Punch***

The leaf punch apparatus is a device that enables investigators to collect DFR samples in a systematic, reproducible manner. A typical leaf punch is a machined tool that consists of a rigid handle to which a trigger mechanism is attached that can depress a round punch of known surface area against a fixed, round blade (Figure B3-3). Three leaf punch sizes are generally available: 1/4, 1/2 and 1-inch diameter punches (i.e., approximately 2.5, 5.0, and 10 cm^2 double-sided surface area). Sampled leaf surfaces are placed between the round punch and the blade. As the trigger mechanism is depressed, round leaf samples of known surface area (herein referred to as leaf punches) are collected for analysis in a vessel affixed to the rigid handle of the leaf punch apparatus below the round blade (e.g., glass jar sealed with a Teflon-lined cap). Leaf punches should be used by investigators whenever possible. Generally, the size and shape of the target crop of interest determines whether or not a leaf punch is feasible for DFR sampling. For example, a leaf punch may not be appropriate for small, oddly shaped foliage. Also, leaf punches may be inappropriate when the active substance is absorbed into the plant fluids. Such fluids become available through the relatively high amount of cuts in the leaves. The advantage of using the leaf punch approach is that samples of known surface area may be collected.

3.2.6.2 ***Whole Leaf***

For scenarios in which the use of a leaf punch is not feasible, whole-leaf samples must be collected. Leaves should be plucked from the target plants using forceps and placed into a sample storage vessel (e.g., glass jar sealed with a Teflon-lined cap).

In cases where whole-leaf sampling is required, a technique must be devised to determine the foliar surface area of each DFR sample. Sample surface areas may be calculated by determining the weight of each DFR sample and multiplying these weight values by a ratio or algorithm that relates surface area to sample weight on a unit basis. (See Part D, Chapter 2.) It is acceptable to make these determinations using fresh control samples prior to sampling in the field if the plant materials used to make the determinations are generally similar to those in the study (e.g., similar maturity leaves). The equipment and techniques used to determine unit target leaf area values are varied. Surface area meters are commercially available that are designed specifically for this purpose. These devices function based on the principle of absorbance, similar to the application of the principle in UV/Vis meters commonly found in most chemistry laboratories. (See Figure B3-4.) Less sophisticated, labor intensive hand techniques have also been used by investigators (e.g., copying representative leaves, cutting out the copied leaves, and relating the weight of the cut-outs to that of the paper on a unit basis).

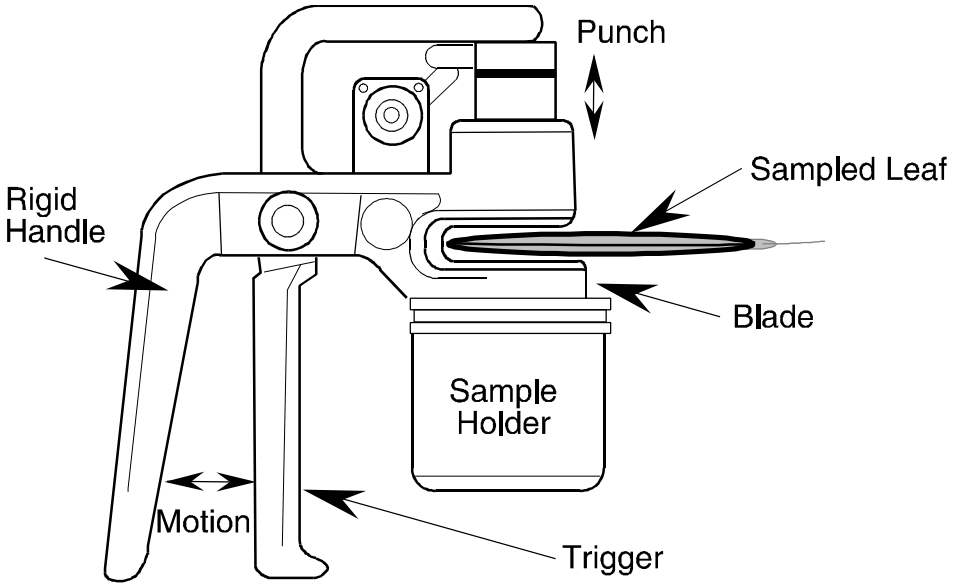

Figure B3-3. Schematic of a Typical Leaf Punch Apparatus

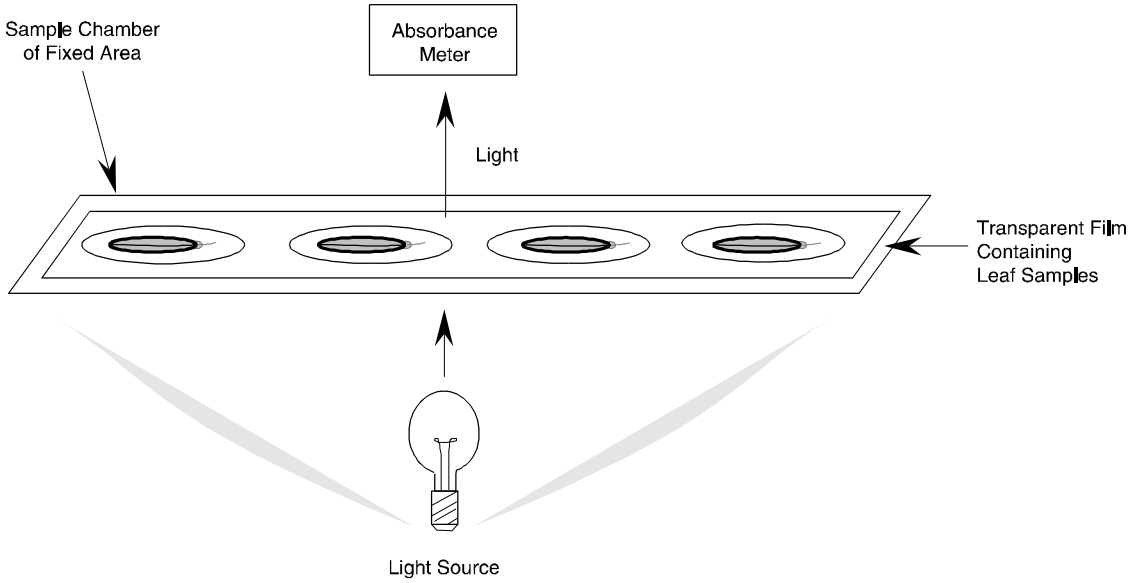

Figure B3-4. Schematic of a Typical Surface Area Meter

Other equipment/techniques may also prove to be viable options for quantifying the relationship of leaf surface area to weight. If an alternative technology is used for this determination, investigators must demonstrate the validity of the technique for determining surface area to weight relationships.

3.2.7 General Considerations for Field Sample Collection

Collecting samples that represent an adequate leaf surface area is critical in any DFR study. Generally, all replicate samples that are collected should represent at least a surface area of 400 cm^2, based on a double-sided leaf area. Double-sided leaf area represents the surface area on both sides of individual leaf-punches or whole-leaf samples (e.g., 1-inch diameter leaf punch represents a double-sided surface area of 10 cm^2). Determination of the required surface area is a simple task if the investigator is using a leaf punch device to collect samples. (See Figure B3-3.) As an example, if a 1-inch diameter leaf punch is being used to collect samples, then at least 40 punches must be included in each sample (i.e., approximately 10 cm^2 x 40 punches/sample). Most leaf punches are equipped with automatic counters to ease the sample collection process. If the whole-leaf sampling method is required for the particular target/crop, investigators must have a knowledge of the surface area to weight ratio for that crop along with some idea of how many leaves represent 400 cm^2 prior to going in the field. The Agency recognizes that investigators using this technique may not always collect samples that represent a sufficient surface area due to the subjectivity involved in sample collection.

Control or background samples should be collected from the test plot prior to application of the test substance. Sufficient control samples should be collected so that fortified dislodging solutions that have been used to wash control leaves can be prepared on each sampling day. These fortified controls should be packaged, transported, stored, and analyzed concurrent with the dislodgeable residue samples. Please see Part C for detailed considerations on Quality Assurance and Quality Control recommendations.

3.2.8 Sample Dislodging Procedures

Dislodgeable foliar residues (DFRs) are those residues present on the surface of a leaf that are available for transfer from the leaf (i.e., residues that have not been absorbed into leaf matrix). DFRs are quantified by washing (i.e., consistently shaking for a set period of time, for example, roughly 10 minutes) chemical residues from the surfaces of leaves using some type of dislodging solution. Generally, the dislodging procedure involves preparing an aqueous surfactant solution, adding an aliquot to each replicate leaf punch sample, shaking the mixture, and retaining the solution for analysis. This procedure should be repeated at least once to ensure a more quantitative residue transfer during the dislodging procedure. The resultant dislodging solutions should be pooled for analysis as one sample. A sufficient quantity of

dislodging solution should be used during each step of the procedure to adequately cover the leaf punches/samples to ensure that the agitation is efficient during the shaking aspect of the procedure.

Aqueous surfactant solutions similar to that proposed by Iwata et al. (1977) are commonly used as dislodging solutions. The procedure proposed by Iwata et al. (1977) was based on the use of an aqueous surfactant solution prepared by adding 4 drops of a 1:50 dilution of Sur-Ten wetting agent into 100 mL of water. Sur-Ten is 70 percent dioctyl-sulfosuccinate sodium salt. A variety of other types of dislodging solutions have also been used (i.e., other surfactants have been used at varying concentrations in different dislodging solution volumes). The Agency has no data to suggest that one aqueous surfactant solution is preferable to another (i.e., at this point any reasonable aqueous surfactant solution is acceptable to the Agency). Investigators have also, in some cases, used organic solvents as dislodging solutions (e.g., ethanol or methanol). However, the use of organic solvents is considered unacceptable by the Agency as these types of solvents may actually extract residues from the leaf matrix itself and not just from the surface of the leaf. Therefore, the results using organic solvents would not be representative of a true DFR.

The Agency recognizes that the use of aqueous foliar dislodging solutions may not be appropriate for all chemicals because of their physical/chemical characteristics (i.e., water solubility). Investigators should consider these characteristics when selecting a dislodging solution.

The determination of an acceptable interval between field sample collection and the actual dislodging procedure is difficult. Therefore, as a protective measure, the Agency recommends that all DFR samples be dislodged within approximately 4 hours of field sample collection (frequently, this is done in the field). This recommendation may be modified on a case-by-case basis, if available data (e.g., environmental fate or product chemistry) or logistical considerations warrant. Most studies that have been completed to date comply with this recommendation. However, several studies have been submitted to the Agency in which the leaf punches were not dislodged for days or even months after sample collection. A study of this nature would most likely not be acceptable to the Agency as the potential exists for DFR absorption into the leaf matrix during storage, thereby, potentially reducing the measured DFR levels.

Another parameter that may significantly impact DFR results is the method of agitation. Historically, two methods of agitation have been employed by investigators: hand shaking and agitation using a mechanical, reciprocal shaking table. The Agency has no data on the advantages and disadvantages of these two methods. Until such data become available to indicate a preference for either method, the Agency will accept the use of either technique in a DFR study. However, investigators must attempt to develop procedures that are as reproducible as possible (e.g., consistently use a reciprocal shaker for 10 minutes at a specified speed or hand shake each sample for 10-minute intervals).

3.2.9 Unit Surface Area Determination

The determination of unit leaf area factors is necessary when the use of a leaf punch apparatus is not feasible. As described above, several types of equipment are available for quantifying this relationship. In all cases, regardless of the equipment/technique selected, the Agency requires that multiple samples be collected and that all analyses be done on a wet weight basis (i.e., extra leaf samples are to be collected for surface area measurements only from the study site). Investigators must ensure that leaf samples used for surface area determinations are not dehydrated, thus altering the relationship between surface area and weight. Preventing dehydration can be accomplished by conducting the surface area analysis within 4 hours (i.e., fresh samples) after harvesting the leaf samples. The surface area determinations may be conducted prior to the study. The Agency requires that a minimum of four replicate samples for at least three distinct theoretical weights be analyzed to define the relationship (i.e., a minimum of 12 samples are required for each surface area to weight analysis). Theoretical weight can be defined as a target weight for preparing each replicate sample, keeping in mind that variations in individual leaf samples will preclude the exact target weight from being met for most samples. However, investigators should attempt to keep actual samples weights within ± 5 percent of the target weight. The three target weights used by investigators should be defined based on the following: 75 percent of the anticipated field weight; the anticipated sample weight; and 125 percent of the anticipated sample weight. The anticipated sample weight can be defined as the anticipated weight of each sample intended for DFR analysis collected in the field (i.e., target weight for field samples that represents the required leaf surface area/sample -- 400 cm^2 per sample, at a minimum, as described above).

3.3 SAMPLE STORAGE

Dislodgeable foliar residue samples and extracts should be stored in a manner that will minimize deterioration and loss of analyte between collection and analysis; more detailed information on sample storage is provided in Part C, Quality Assurance and Quality Control. Also see Residue Chemistry Methods 860.1340 (Subdivision O) for more information. The study investigator is responsible for demonstrating the stability of the samples under the storage duration and conditions used.

3.4 SAMPLE ANALYSIS

Pesticide residues should be dislodged from leaves within 4 hours of sample collection. Validated methods of appropriate or sufficient sensitivity are needed for all sample analyses. See Part C, Quality Assurance and Quality Control for more detailed information on sample analysis.

3.5 CALCULATIONS

Refer to Part D of this document for a description of the calculations needed for estimating dissipation rates, exposure, and risk.

3.6 DATA PRESENTATION

Dislodgeable foliar residues should be reported as mg or µg of pesticide active ingredient per m^2 or cm^2 of leaf sampled. These data should be reported in tabular form for each sampling day. In addition, the best fit dissipation curve should be plotted (typically log-linear) with dislodgeable foliar residues on the Y-axis and time on the X-axis. Distributional data should be provided, to the extent possible.

REFERENCES FOR PART B, CHAPTER 3

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Iwata, Y.; Knaak, J.B.; Spear, R.C.; Foster, R.J. (1977) Worker Reentry into Pesticide Treated Crops, In: Procedure for the Determination of Dislodgable Residues on Foliage. Bull. Environ. Contam. Toxicol. 18:649-655.

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