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GUIDANCE FOR DETERMINATION OF DISLODGEABLE FOLIAR RESIDUE

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INTRODUCTION

This document provides information and guidance for designing and conducting studies, primarily dissipation studies of foliar pesticide residues, and analyzing the resulting data to quantify dislodgeable foliar residues (DFR) that may be available for field worker exposure. DFR samples can be used in numerous ways, these include: (a) determining the dissipation rate of the pesticide; (b) assist in determining the environmental load of the pesticide; and (c) estimating the exposure potential of field workers. This document discusses the rationale for conducting a study, study design, sample collection, and provide general information on analytical methods and detailed information on data analysis used to quantify DFR.

For the purpose of this document, DFR is the pesticide residue that can be removed by washing the surface of the leaf with a water/surfactant solution. A field worker is any person taking part in manual cultural activities required for the production of an agricultural commodity.

Researchers conduct DFR studies when: (a) the toxicological properties of the pesticide or the pesticide product cause concern for field worker safety; (b) the pattern of use or the timing of the application relative to worker reentry cause concern for field worker safety; or (c) data on field worker exposure are needed, but not available. DFR dissipation data along with an appropriate transfer factor[1/] can be used to calculate surrogate estimates of dermal exposure.

STUDY DESIGN

The study will be conducted under the guidance of a single study director. All staff collecting samples must review the study protocol and receive thorough instructions in pertinent Standard Operating Procedures (SOPs), equipment use and in leaf selection criteria (See the *Sample Collection* section of "Sampling Strategies for Dissipation Studies – Leaf Discs", below).

The utility of DFR samples is contingent on collecting samples that are representative of the pesticide treated areas. Representative samples (characteristic of the population) are necessary to accurately measure the DFR. To obtain representative samples, researchers must clearly identify the population, experimental unit, treatment and sampling unit.

Definitions

Steele and Torrie[2/] offer the following definitions:

Population - All possible values of a variable.

Experimental Unit - The unit of material to which one application of a treatment is applied.

Sampling Unit - Some fraction of the experimental unit upon which the effect of the treatment is measured.

Treatment - The procedure whose effect is to be measured and compared to other treatments.

In general, the term *replicate* refers to an independent repetition of the complete experiment (Milliken and Johnson[3/], Neter *et al*.[4/]). That is, if there are *k* treatments, a replicate consists of *k* experimental units that are "identical" except for the treatment applied. Independent repetition in a DFR study requires, minimally, a new tank mix. If multiple sites or applications are monitored under similar conditions, the sites or applications are the *replicates*. Replicates provide an estimate of experimental error, improve precision, increase the scope of inference, and account for error variance. In describing the study, the term *sample* should be used for the samples taken each day.

Determination of Population

Defining the appropriate population depends on the purpose of the study. If the main focus is to understand the dissipation of particular pesticide, the population includes all California crops treated with that pesticide, all areas where the crops are grown, and all seasons when the pesticide is applied to those crops. If the focus is dissipation of a selected pesticide on a particular crop, the population of interest is that one crop.

If pesticide applications are typically made at different times of the year, consideration should be given to conducting studies during each of those periods for comparison of the effects of environmental conditions, plant growth, etc. A study conducted by Reeve and O'Connell[5/] demonstrated an increasing half-life for applications of methomyl on grapes made from April through October. A DFR dissipation study should be conducted at the typical time of high pesticide use. If a pesticide is normally applied in the spring, studying an application made in the fall might not provide information useful for characterizing potential worker exposure for the majority of the pesticide use. On the other hand, if 50% of the pesticide is used in April and 50% used in July, studies should be conducted during both of the major use periods to fully characterize residue behavior.

Selection of Experimental Units

Once the population is determined, the researcher must select the experimental unit(s) or study site(s) that are representative of the population. (When there is only one treatment, i.e., no comparisons are being made, the study site and the experimental unit are the same. When there is more than one treatment, each study site may have an experimental unit for each treatment). The researcher needs to identify the factors that vary between units in the population and that may potentially affect deposition, dissipation and/or worker exposure. Experimental units should be selected representing the range of values of these variables, so that the study conclusions will be broadly applied.

Researchers should base the choice of study sites on the pesticide use patterns, crop (heavy pesticide use, potential for significant worker exposure, etc.), timing of pesticide applications and location (relative to climate, soil type, etc.). Differences in application rates, techniques or patterns may necessitate sampling at several sites to fully characterize residue deposition and behavior to learn the effects of the application parameters.

At the discretion of the study director, an untreated control field could be set up and sampled in the same manner as the treated field(s) to measure baseline properties.

Selection of Sampling Units for DFR Dissipation Studies

The following presents an overview of the steps needed to properly select sampling units within the study site(s). This document cannot cover all possible scenarios that the researcher may encounter in field situation; but provides general information to aid in the selection of study sites.

- Sketch the complete experimental unit; include roads, direction of rows, field irregularities (low spots, differences in crop growth, missing plants, etc.).
- Since field borders often have a wide variation in application rates and in environmental conditions, these areas should not be included in the sampling scheme for DFR degradation studies, unless that area is of particular interest. A border of at least 20 feet (6 meters) should be adequate for most treated areas.
- Overlay a grid system on the field. Each section of the grid is one potential sampling unit. The size of the units will depend upon what is required to obtain a representative sample. For example, this might be 1-2 rows wide and 15-25 feet (3-5 meters) long.
- Randomly select units for sample collection. This can be accomplished by numbering each unit and randomly (random number generator) selecting sampling units. Identify the sampling unit selected in the field (flagging tape, stakes, etc.) so that repeated measurements can be collected.
- Four to six sampling units are recommended from each study site. Gunther et al.[6/] and Iwata et al.[7/] have shown residue variation of approximately 15% with three samples per site.

Randomization in the selection of sampling units helps to protect against sources of bias that are unsuspected[8/]. For example, if a pesticide application device treats three rows at a time and the researcher happens to select every third row to sample, then a bias exists for the rows sprayed with the same nozzles. If one of those nozzles is not functioning properly, then the researcher is probably not obtaining samples representative of the experimental unit.

Sampling Interval

The length of time from the first to last sampling interval will depend upon the expected level of residue at the time of first sampling (estimated), the expected half-life (estimated), and the limit of detection for the residue under study (known). Ideally, one should select sampling intervals that will show the same decrease in magnitude of DFR from one sampling interval to the next. However, even with a basic understanding of the pesticide's physical and analytical parameters, it is difficult to pinpoint the ideal sampling intervals. WH&S studies have shown that sampling over a period of time equal to six half-lives (approximately 98% dissipation/loss) is adequate for refining the original estimate of residue half-life, and is narrow enough to expect that samples will still be within the laboratory limit of quantification/detection.

As a guideline, each sampling interval should be approximately double the previous interval. Sampling should initially be conducted more frequently, with intervals lengthening with increasing time post-application. The last samples should be collected just before the minimum detectable level would be reached. Generally, if several fields are considered for study, the

researcher will approach experimental design based on the initial assumption that DFR in all fields will behave similarly and thus apply a single sampling strategy to all fields.

A set of samples should be collected immediately before the application using the same sampling method as post-application samples.

SAMPLE COLLECTION

Selection of leaves

The most important factor in leaf selection for sampling is consistency. Significant variation can be introduced through inconsistent sample collection. Staff should collect all samples for a given study according to **explicit leaf selection criteria.** These criteria should be specified in the study protocol.

As a rule, the investigator should ensure that the portion of the leaf to be punched is not handled before sample collection. Do not handle the discs once they have cut from the leaf. After collecting each individual sample, staff should thoroughly clean the leaf punch with water and paper towels, making sure to remove all plant residue from the cutting die. This will prevent both cross-contamination of the subsequent sample and punch malfunction due to a build-up of plant matter on the cutting die.

The actual plant location where the leaves or discs are collected depends upon the objective of the study and is generally specified in the study protocol. Within study parameters, leaves must then be randomly selected in order to achieve representative samples across the sampling unit. Collect samples from areas of the plant where worker contact might be expected.

Sample fully mature leaves; residues on young leaves are subject to growth dilution and may influence the measurement of DFR. In addition, sample only the leaves that were most likely on the plant at the time of the treatment with the pesticide in question, making sure to avoid new growth.

The leaves should be free of excess surface moisture when sampled.

In most cases, use the following guidelines adapted from Iwata *et al.* (1977):

Tree foliage- Sample at a height of 4-6 feet (1.5-2 meters), unless resources and field conditions allow for sampling of the entire canopy. Spencer et al.[9/] found some variation in initial deposition but not in residue half-life when samples were collected at three heights in trellised apple tree canopy. Two to four leaf discs should be collected from each tree while ensuring that throughout the sampling unit all sides (four 90º positions around the circumference) of the trees are sampled.

Vine Crops- Sample near the location of a vine that workers would be expected to contact, with one to two discs collected from each vine. Welsh et al.[10/] found differences in initial deposition and dissipation in samples collected from the outside of the canopy vs. those inside the canopy.

Row Crops- For most row crops, sample the outside (i.e., wrapper) leaves with one disc collected from each plant sampled. Ensure that discs collected represent all sides of the plants.

Sample Collection Methods for Leaf Discs

To date, leaf discs are the method of choice for investigators. The discs are easy to collect using manufactured leaf punch devices and can be efficiently extracted and analyzed. Ideally, use a leaf punch that cuts a disc with an diameter of at least 2.5 cm, or the largest possible considering leaf size and shape. Smaller discs have a larger cut surface to disc surface area ratio that may influence the measurement of DFR[8/]. However, some leaves such as celery, tomato and carnation are too small to use a 2.5 cm diameter leaf punch. For these types of crops, use either leaf punches equipped with a 1.25 cm cutter, or use whole leaves and the Li-cor® LI-3100 Area Meter to measure the total sample size. (See next section)

Sample Size Gunther et al.[6/] and Iwata et al.[7/] suggest that a minimum of 40 2.5-cm diameter leaf discs (or approximately 400 cm² of double-sided leaf surface area) be collected per sample. Forty leaf discs will fill a 4-oz. jar (usually the size that attaches to manufactured leaf puncher) and still allow sufficient capacity for washing the leaf discs. If the leaf disc count is not documented during collection, save the discs for a count once the wash is complete.

Sample Containers The sample container must be compatible with the leaf puncher, the mechanism used for washing the sample, and the pesticide under investigation. In general, a clear and clean 4-oz. (48mm diameter) glass jar is used. However, some pesticides adsorb to glass and another type of material will be necessary as a container.

Sample Collection Methods for Whole Leaves

Whole leaves are usually collected only when a leaf punch is unavailable, if foliage is irregularly sized or too small for the diameter of the puncher. When collecting whole leaves, the sampling staff must ensure that they do not contact the leaf surface with their hands or sampling tools. The Li-cor® LI-3100 Area Meter machine is ideal for accurately measuring total sample surface area of whole leaves. (Reference the Li-cor® LI-3100 Area Meter Standard Operating Procedure: WHS-EQ06)[11/]. Keep in mind that the total measured area should not exceed the limits set forth in the study protocol. The DFR database for extraction of residue from whole leaves is limited (Smith et al.[12/] and Bissell et al [13/]).

Sample size The number of leaves collected per sample should adequately represent the treated foliage within the sampling unit. Generally, the number of leaves collected should represent a minimum total area of 400 cm^2, but depends on study protocol. Sample size for whole leaf sampling must also take into account the size of the leaves and still be representative of the sampling unit.

Sample containers The sample container must have adequate capacity for the leaves collected and for the washing process during extraction. Collect leaves in the same container as will be used for washing the resides from the leaf surface. Do not transfer leaves from one container to another, as some of the surface residue may be lost. The study director should consult the laboratory staff for guidance in selecting appropriate sample containers.

Sampling Strategies to Evaluate Potential Worker Exposure Following an Illness Incident
In general, collection of samples to evaluate worker exposure potential following an illness incident involves a one-time sampling event. Collect leaf discs from areas of the plant where worker contact is expected; this would include old *and* new leaves. Collect samples from an area of the field not touched by workers. For example, if a 20-acre field was treated and workers harvested the south 10 acres, collect samples from the north 10 acres. Mark the areas as sampled; we may need to re-sample at some future date to determine if it is safe to reenter.

Collect samples from all fields the workers entered (working or resting). Take an appropriate number of samples to give a representative picture of potential exposure. As a general guide use the same recommendation discussed above, 4 to 6 samples per field.

It is imperative to get these samples analyzed as quickly as possible as we may need to determine if a continuing hazard is present.

Samples should be stored and shipped in the same manner as all DFR samples; see discussion below.

Sample Storage and Shipment to the Laboratory
Immediately following sample collection, tightly seal all samples. For glass jars, seal with Teflon®-lined lids or cover with aluminum foil and an unlined lid. Double bag jars in resealable plastic bags and seal. Place the bagged samples on ice. **Do not store on dry ice or freeze. (**Freezing ruptures the leaves' cell walls, and the resultant leakage of plant fluids may influence the measurement of DFR.) If the samples will be sent via overnight postal or bus service, place bagged samples on ice in a chest. Seal the ice chest openings with heavy-duty tape several times so that the ice melt stays contained

Extraction of DFR should be accomplished within 24 hours of sample collection. Storage for more than 24 hours may affect the amount of residue that is dislodgeable. Document the time of storage and other pertinent sample collection information on the chain-of-custody form. Standard Operating Procedure for the chain-of-custody is delineated in a Memorandum dated August 20, 1998 entitled "COC Protocol."[14/] Also refer to the SOP in WHS-FO04 entitled "Identification and Labeling of Samples."[15/]

For proper compliance with delivery of samples to California Department of Food and Agriculture (CDFA) Center for Analytical Chemistry, Worker Health and Safety Laboratory, reference the Standard Operating Procedure WHS-FO05 entitled, "Sample Tracking, Shipping and Receiving"[16/] which explains the reporting requirements. In addition, all samples must be turned in before noon on the last work day of the week according to the March 2, 1998 MOU entitled "Friday DFR Sample Delivery."[17/]

TANK MIX SAMPLES

If it can be collected safely, a sample of the actual pesticide mixture applied to the experimental unit should be collected from the application tank after it has been thoroughly agitated. This may assist the investigator in evaluating the effect of application rate on initial deposition and dissipation rate. It may also enable some comparison between the theoretical and the actual application rate. However, tank mix samples should be considered qualitative. Research[18/] has shown considerable variation in tank mix sample analyses following precise measurements of the amounts added to the spray tank.

If the researcher cannot collect a tank mix sample, they may substitute observation of the mixing procedure and a sample of the formulated product. With these two items, the researcher can qualitatively verify that the proper amount of material was mixed into the spray tank.

The tank mix or formulation samples should be sub-sampled, then stored on dry ice or frozen until analysis. Store these samples separately from DFR samples. Refer to appropriate standard operating procedures for sample collection techniques, documentation, chain-of-custody, storage and shipment procedures and record keeping.

RECORDS

Record all details of the application process including: amount of pesticide put into the tank, application rate, method of application, amount of diluent added to tank, other components in the tank mix, verification that the proper amounts were added to the tank, application time, environmental conditions, etc.

The investigator should note field conditions when sampling, any cultural activity that has taken place in the field, environmental conditions during the course of the study, sample collection date and time, etc. Environmental data can be collected via portable weather stations or through the California Air Resources Board (air quality data), the University of California CIMIS database or the California Department of Water Resources (weather data).

See Appendix 1 for a sample of record keeping forms. Refer to the appropriate standard operating procedures for record-keeping requirements.

LABORATORY ANALYSIS

All policies and procedures regarding laboratory analysis of samples are guided by CDFA. Samples to be analyzed for DFR should be washed within 24 hours of collection. Failure to extract within 24 hours may influence the measurement of DFR. Avoid the use of organic solvents as they may carry surface residue into the leaf tissues or extract penetrated residues.

Leaf Discs
The following general technique is often used to wash residue from the leaf surface:

To the sample collection container holding the leaf discs (usually a 4-oz. jar), add 50 mL of distilled water and 0.2 mL of a 3% solution of sodium dioctyl sulfosuccinate. Rotate jar for 20 minutes and decant the aqueous solution into a separatory funnel. Repeat the washing procedure twice more for a total of three washings. Add sodium chloride to the separatory funnel and shake to dissolve. Extract the aqueous solution with the appropriate solvent and drain the solvent through glass wool and sodium sulfate. Repeat this procedure twice, combining all three extracts.

Analyze the extract using equipment and procedures appropriate for the pesticide in question.

Whole Leaves
To the sample collection jar, add the required amounts of distilled water and sodium dioctyl sulfosuccinate solution. (For 10 leaves use about 400 mL of water; for 20 to 40 leaves use about 800 mL of water.) Place jar on a shaker table for 30 minutes. Decant the aqueous solution into a separatory funnel. Add sodium chloride to the separatory funnel and shake to dissolve. Extract the aqueous solution with the appropriate solvent and drain the solvent through glass wool and sodium sulfate. Repeat this procedure twice, combining all three extracts.

Analyze the extract using equipment and procedures appropriate for the pesticide in question.

CALCULATIONS AND PRESENTATION OF RESULTS

This section outlines the policy of the Worker Health and Safety (WH&S) Branch with respect to the statistical treatment of DFR data. *For further detail, reference HSM-00011.*[19/]

Data Preparation
Nondetects (ND) When a set of postapplication samples from a single sampling unit and interval has a mixture of both detected and non-detected residues, substitute one-half the limit of detection (LOD) for any sample with no detected pesticides. If any samples from the last days of sampling are ND, drop the day(s) from the analysis. In other words, use only the data through the last day with any detects.

For background (preapplication) samples, substitute zero if all samples taken the same day under the same conditions were ND.

WH&S Exposure Monitoring Program does not generally apply any correction for percent analytical recovery unless specified otherwise in study protocol.

Arithmetic(day) mean of samples for each day For each sampling unit and interval, calculate the arthmetic mean of the sample results (day mean). This "day mean" is the basis for summary statistics, data transformations, and statistical analyses. Generally, the logarithm (either base 10 or base *e*) of the arithmetic (day) mean will be taken for the purpose of statistical analysis.

Correction for background To estimate initial deposition, subtract the mean background residues present on the preapplication samples from the mean of the Day 0 sample residues. Logarithms of this difference can then be taken. If pre-application residues were not sampled, estimate the initial deposition from the intercept of the dissipation curve.

For the purpose of estimating DFR dissipation, samples are not corrected for background.

Statistical Analysis.

Means and standard deviations (S.D.) When simple means and standard deviations are presented, they should be the arithmetic statistics, calculated on the untransformed variable (i.e., not on the logs). This is true even when the variable is thought to be lognormally distributed and logs are used in the regression analysis. (There are better ways to estimate the mean and standard deviation of a lognormal distribution, but they are slightly complicated. You may consult a statistician to do these calculations.)

Confidence intervals (CI) For normally distributed variable. The familiar formula,

$$\text{Arithmetic Mean} \forall\ t_{(.975;\ n-1)} * (\text{S.D.}/\%n),$$

is valid for the 95% confidence interval for the mean.

Ordinarily, however, we will assume that DFR is lognormally distributed. The 95% confidence interval for the mean can be found in either of two ways. One is by calculating the arithmetic mean and S.D. of the logs, substituting them in the previous formula, then taking the antilog of the result:

$$\text{antilog}\{\text{Arithmetic Mean of logs} \forall\ t_{(.975;\ n-1)} * (\text{SD of logs}/\%n)\}.$$

Alternatively, the CI can be calculated from the geometric statistics:

$$\text{Geometric Mean} * (\text{Geometric SD}/\%n)^{\forall\ t_{(0.975;\ n-1)}}.$$

Dissipation curve The log-linear regression model,

$$log\ \text{DFR} = ß_0 + ß_1 * (\text{days}),$$

or the log-quadratic model,

$$log\ \text{DFR} = ß_0 + ß_1 * (\text{days}) + ß2 * (\text{days})^2,$$

should be fit to the log of mean DFR for each day (as described above). If there are replicates (e.g., multiple applications), they are analyzed in one regression analysis. The simpler log-linear model may be used if it adequately describes the data. The log-quadratic model should be used if adding the days-squared term increases R^2 by 0.05 or more over the log-linear model (if you know how to do a stepwise regression, you can use that technique to decide whether to include days-squared; in 26 DFR datasets, the 0.05 rule of thumb gave a good approximation to the results of stepwise regression with the significance level to enter at 0.10). Occasionally it may be necessary to consider other models. If neither model fits well (significance *p* for overall model > 0.05) or the results seem anomalous, consult a statistician.

Half-life may be reported, but it will generally be more meaningful to give a table of predicted DFR by day after application. Predicted DFR in µg/cm² should be calculated by an unbiased backtransformation of the predicted log (Powell[20/]). The table should give predicted µg/cm² for every day from Day 0 through the last sampling day used in the regression analysis. Normally, prediction should not be extrapolated beyond the last sampling day used in the regression, but exceptions may be made if a specific day after application or a specific DFR level are of interest and lie beyond that day. Prediction limits (usually only the one-sided upper limits) should also be given in the table. Prediction limits are similar to confidence limits, but they apply to individual replicates rather than to the mean.

When the quadratic model is used, predicted DFR may begin to increase at some point. This can happen because of the nature of the quadratic model and/or random variation in the data. (You should consult a statistician to make sure the model is fit correctly.) In such cases, the lowest value reached by predicted DFR will be used as the predicted value for subsequent time points.

Some DFR studies compare deposition and dissipation under different conditions, for example, inside and outside the canopy, or with different application methods. For these studies, more complex regression models are required, and a statistician should be consulted.

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Appendix 1

FIELD RECORD KEEPING FORM

Study Identification # _____

Pesticide Identification
Trade Name _____
Common Name _____
EPA Registration # _____
Formulation _____
Action _____

Reentry Interval
Federal _____
California _____
Preharvest Interval _____

Application Information
Application Technique _____
Equipment: Make/Model _____
Nozzle Configuration _____ PSI _____
Rate (lb a.i./acre) _____ Diluent _____
Mix concentration _____ Time to Apply _____
Temperature: Start _____ Completion _____
Other materials in tank:
Name _____ Rate (lb a.i./ac) _____
Name _____ Rate (lb a.i./ac) _____
Name _____ Rate (lb a.i./ac) _____
Name _____ Rate (lb a.i./ac) _____
Name _____ Rate (lb a.i./ac) _____

Name of Applicator _____ PCO PCA Grower Other
(circle one)

Commodity/Field Information
Commodity _____ Variety _____
Ranch Name _____ Block No(s) _____
Size of Treated Area _____ Crop stage _____

Row Direction (circle one) N S E W
Type of Irrigation _____
Field Condition (weedy, dusty, etc.) _____

Owner/Contact Person _____ Phone_____

Sketch of Field *(Label which areas of the field were sampled)*

Appendix 1

SAMPLING INTERVAL INFORMATION

Study Identification #_____

Sample Numbers	Date	Day Post-Application	Time	Field Notes (Cultural practices, irrigation, etc.)