

PROTOCOL FOR  
TERRESTRIAL FIELD DISSIPATION  
OF <TEST SUBSTANCE> AND ITS METABOLITES FOLLOWING  
APPLICATION OF <TEST SUBSTANCE> TO BARE SOIL  
IN <STATE(S)>

EPA Guideline Requirement

FIFRA GUIDELINE NO. 164-1  
OPPTS GUIDELINE NO. 875.2200

Protocol Number



Prepared By:

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**PROTOCOL FOR TERRESTRIAL FIELD DISSIPATION  
OF <TEST SUBSTANCE> AND ITS METABOLITES FOLLOWING  
APPLICATION OF <TEST SUBSTANCE> TO BARE SOIL  
IN <STATE(S)>**

**SPONSOR:**

<Name>  
<Address>  
<Phone>

**TESTING FACILITY:**

LANDIS INTERNATIONAL  
3185 Madison Highway  
Valdosta, GA 31603-5126  
Phone 229-247-6472

**STUDY DIRECTOR:**

<Name>  
LANDIS INTERNATIONAL  
3185 Madison Highway  
Valdosta, GA 31603-5126

**ANALYTICAL TESTING FACILITY:**

<Name>  
<Address>  
<Address>  
<Phone>

**STUDY MONITOR:**

<Name>  
<Address>

**PROTOCOL NUMBER:**

Trial No.	Location (EPA region)	Field Cooperator

**TEST SYSTEM:** Bare soil

**TEST SUBSTANCE:**

<Pesticide Trade Name>  
**Formulation:** <Percent active ingredient, type>  
**Lot No.:** Will be recorded in the study file  
**CAS No.:** <Number>  
**CAS Name:** <Name>  
**Common Name:** <Name>  
**EPA Reg. No:** <Number>  
**IUPAC Name:** <Name>

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IN <STATE(S)>**

**PROTOCOL RECIPIENTS:**

- |                            |                                      |
|----------------------------|--------------------------------------|
| (1) Sponsor                | (4) Field Cooperator                 |
| (2) Study Director         | (5) Analytical Laboratory            |
| (3) Quality Assurance Unit | (6) Soil Characterization Laboratory |

**GLP NOTEBOOK RECIPIENT:**

- (1) Field Cooperator

**SAMPLE ACTIVITY I.D. LOG RECIPIENTS:**

- |                      |                                      |
|----------------------|--------------------------------------|
| (1) Sponsor          | (4) Analytical Laboratory            |
| (2) Study Director   | (5) Soil Characterization Laboratory |
| (3) Field Cooperator |                                      |

**PROPOSED EXPERIMENTAL START DATE:** <Date>

**PROPOSED EXPERIMENTAL COMPLETION DATE:** <Date>

## OBJECTIVE

The purpose of this study is to determine the extent and rate of dissipation and the vertical mobility of <test substance> and its metabolites in the soil profile under typical use when applied to bare soil at the maximum seasonal rate of <amount> lb ai/A <test substance> (<number> applications at <amount> lb ai/A per application at all sites).

## JUSTIFICATION OF TEST SYSTEM

**Application:** Various commercial crops will be treated with <test substance> for control of <target pest>. A maximum seasonal application rate of <amount> lb ai/A will be applied by using <number> separate ground applications (<amount> lb ai/A in each application) at a <number> day interval on bare soil at all locations. This is the maximum labeled rate. Bare soil will be used in order to eliminate crop interference and assure uniform application.

**Sampling System:** The proposed application and sampling methods have been successfully employed to determine soil residues of agricultural chemicals for past studies. The devices used for sampling minimize contamination in samples from lower soil horizons that may occur from treated soil from higher horizons being distributed throughout the profile during the sampling process. The sampling frequency is based on a prior general knowledge of the degradation of <test substance> in soil.

## IDENTIFICATION OF TEST SYSTEM

**Experimental design:** The study site will consist of 4 plots (3 replicate treated plots and 1 non-treated control plot) established where no <test substance> (or pesticide known to interfere with <test substance> or its metabolites) has been applied for at least 5 years. The plots will be designed using the guidelines in LANDIS INTERNATIONAL SOP #3.2 - Current Revision, *Plot Construction and Identification* (see GLP Field Notebook provided by LANDIS INTERNATIONAL, INC.).

**Plot size:** The 3 treated plots will each have a minimum area of 1200 ft<sup>2</sup> or the minimum size required to collect the required number of samples. The non-treated plot will be at least the same dimensions as one of the treated plots and must be a minimum distance of 150 ft upslope and upwind (slope direction supersedes wind direction) from the nearest treated plot. Wind direction refers to prevailing wind; however, the non-treated plot must not be downwind from the treated plots at the time of application.

The experimental design proposed conforms with the U. S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision N - Chemistry: Environmental Fate (§164-1) studies and OPPTS Guideline 875.2200.

## APPLICATION

**Test substance:** The date of test substance shipment, lot number, date of receipt, and method of shipment, as well as the amount and container size, will be documented. The test substance will be stored in an appropriate manner and storage conditions and daily temperature extremes will be recorded. Subsequent to the final application of test substance, the remaining <test substance> will be returned to LANDIS INTERNATIONAL, INC.

The Sponsor will obtain a purity analysis and characterization for each lot number of the test substance used. Information regarding the stability and homogeneity of the test substance will be part of the study raw data package. A retention sample from each batch of the test substance will be archived by the sponsor. All unused test substance and partially empty containers will be retained until the final report is signed, unless a prior waiver has been obtained from EPA. Test substance remaining at the completion of the study will be returned to <Sponsor>.

**Application:** The soil in the plots must be moderately moist at the time of each application. The test substance will be applied as a broadcast ground treatment according to the details listed below:

Test substance:	<name>
Formulation:	<formulation>
Carrier:	water
Rate:	<lb ai/A and amt. product/A>(± 10%)
Spray volume:	<volume>
Number of applications:	<number>
Timing:	<spray intervals>
Application equipment:	<type of sprayer/nozzle, etc.>

The application equipment will be calibrated according to appropriate SOPs prior to treatment. Calibration results will be sent to the Study Director after each application. Complete documentation will be recorded in the field logbook.

The actual application rate will be calculated based on output, the active ingredient concentration, and the application time or land area covered. Once the plot has been treated, the amount of product or spray volume will be checked and recorded as verification of the application rate. The remaining product material will be disposed of appropriately and in accordance with federal and state regulations.

## PLOT MAINTENANCE

Plots will receive overhead irrigation to meet a selected crop coefficient value or the 30-year monthly average precipitation plus at least 10% (whichever is greater) throughout the study. Deficits from the previous month will be carried over into the calculation for the current month. However, surplus precipitation will not be carried over to the current month. The amount of irrigation water and timing will at least be a typical amount to grow a target crop.

Herbicides and/or mechanical means that do not disrupt the soil surface will be utilized throughout the experiment to prevent weed growth. The Field Cooperator will ascertain that all maintenance pesticides will not interfere with the analysis of acequinocyl or its metabolites before they are used in the test plots. Each maintenance pesticide will be approved by the Study Director before it is applied to the test system.

Holes resulting from sampling of soil will be marked with a flag or stake at the time of sampling and refilled on the same day with soil free of any residues of the test compound or interfering substances.

## SAMPLING

**Site Qualification Soil:** Prior to the anticipated initial application date, *<number>* soil samples, consisting of 36" soil cores in acetate probe liners, will be collected randomly from within the study area. *<Number>* samples will be taken from each of the areas designated as treated and *<number>* from the non-treated plot. The cores will be segmented by the cooperating Field Study Scientist into the following increments: 0-6", 6-12", 12-18", 18-24", and 24-36". Red caps will be placed on the upper end of the section and black caps on the bottom. The segments from like depths within a plot will be taped together in their liners and shipped frozen for analysis to substantiate there are no substances in the soil that interfere with analysis of *<test substance>*. Samples will be shipped as soon as possible via an overnight courier service and packed with sufficient dry ice to keep the samples frozen for at least 48 hours (LANDIS INTERNATIONAL SOP #4.14-Current Revision, *Packing/Shipping Sprayate Samples, Petri Plates, Field Spikes, Soil Cores and Crop Residue Samples*).

**Soil Characterization Samples:** Before the conduct of the study, soil samples will be collected to a depth of 36" for characterization analysis. At least *<number>* cores will be collected from each of the *<number>* plots and core segments for each depth interval for all plots will be combined as a composite sample; a 1-kg subsample from each depth interval will be selected for shipment to the laboratory. The depth intervals will be: 0-6", 6-12", 12-18", 18-24", and 24-36". The samples will be shipped to:

SOIL CHARACTERIZATION LABORATORY

*<Name>*

*<Address>*

*<Address>*

**SAMPLING (CONT'D)**

The soil will be analyzed for the following six parameters:

- |   |                                |
|---|--------------------------------|
| (1) Acidity (pH)                                    | (5) Bulk Density               |
| (2) Cation Exchange Capacity (CEC)                  | (6) Particle Size Distribution |
| (3) Organic Matter (OM)                             |                                |
| (4) Water Holding Capacity (1/3 and 15 bar tension) |                                |

**Biomass Samples:** At <number> intervals <number> cores will be collected in a representative manner from each plot to a depth of at least 6" and composited. Proposed intervals are prior to first application, at the 120 day interval and at the 540 day interval or after the last sampling interval. A subsample of at least 1 kg will be collected from each composited sample and shipped to the analytical laboratory at ambient temperature for biomass analysis.

**Field Spikes:** The analytical laboratory will provide pre-measured samples of <test substance> and its metabolites for mixing with nontreated soil from each test site. The spiked soils will be utilized for confirming the stability of residues during storage and shipping. The laboratory will provide complete instructions for preparation of the samples to the Study Director for review and approval prior to shipment of the pre-measured samples to the Field Study Scientist. Two sets of field spikes using 0-6 inch segments will be prepared to coincide with two sampling events during the course of the study. These samples will be shipped via overnight delivery service to <Analytical Laboratory> along with the group of test samples collected at the designated interval. At the Study Director's discretion, only one spiking interval may be performed. Reference LANDIS INTERNATIONAL SOP #4.10-Current Revision, *Shipping Stability Samples* in the GLP Field Notebook for further guidance in the handling of samples.

**Soil Residue Samples:** Sampling for <test substance> persistence and leaching will be done from the treated plots at the approximate intervals shown in the following table (Sampling Intervals). Actual dates of application and sampling may vary within several days from dates in the "Sampling Intervals" Table seen below. Nontreated samples will be collected at the -35, -21, -16, 0, 1, 7, 15, 30, 60, 90, 120, 240, 360, 450, and 540 day intervals at the same depth(s) as treated samples.

ALL OPERATIONS AND ACTIVITIES WILL BE PERFORMED IN THE NON-TREATED CONTROL PLOT FIRST AND THEN IN THE TREATED PLOTS. SAMPLING TECHNIQUE AND SAMPLE HANDLING FROM THE TIME OF SAMPLING UNTIL PLACING SAMPLES IN THE FREEZER WILL BE FULLY DOCUMENTED.

**Soil Core Samples:** The following soil increments will be taken as described below:

1. 0 - 6" depth - taken using > 2" OD (outside diameter) acetate (butyrate) tube.
2. 6 - 36" depth - taken using a 7/8"-2" OD acetate tube. This tube and the enclosed soil will be sub-sectioned by the Field Study Scientist into the following increments: 6-12", 12-18", 18-24", and 24-36".

**SAMPLING (CONT'D)**

EXAMPLE FOR TWO APPLICATIONS SEPARATED BY 21 DAYS:

SAMPLE TIMING* (DAYS)	PLOTS TO BE SAMPLED	SAMPLE DEPTH (INCHES)	APPROXIMATE DATE
-35 (prequalification)	TRT & NTRT	0-36	<DATE>
-21 (before app. 1)**	TRT & NTRT	0-6	<DATE>
-21 (3 hr. after app. 1)	TRT & NTRT	0-6	<DATE>
-19	TRT	0-36	<DATE>
-16	TRT & NTRT	0-36	<DATE>
-10	TRT	0-6	<DATE>
0 (before app. 2)**	TRT & NTRT	0-36	<DATE>
0.125 (3 hr.)	TRT	0-6	<DATE>
1 (24 hr.)	TRT & NTRT	0-36	<DATE>
3	TRT	0-36	<DATE>
5	TRT	0-36	<DATE>
7	TRT & NTRT	0-36	<DATE>
11	TRT	0-36	<DATE>
15	TRT & NTRT	0-36	<DATE>
30	TRT & NTRT	0-36	<DATE>
60	TRT & NTRT	0-36	<DATE>
90	TRT & NTRT	0-36	<DATE>
120	TRT & NTRT	0-36	<DATE>
240	TRT & NTRT	0-36	<DATE>
360	TRT & NTRT	0-36	<DATE>
450	TRT & NTRT	0-36	<DATE>
540	TRT & NTRT	0-36	<DATE>

\* Sampling may be terminated earlier than planned at the discretion of the Study Director if residues dissipate below the LOQ prior to the next sampling date. If samples at certain intervals cannot be taken due to frozen ground or snow, samples will be taken as soon as weather permits.

\*\* Samples scheduled to be taken prior to each application may be taken on the day preceding the application, if necessary.

### SAMPLING (CONT'D)

The soil probe with an acetate tube and metal sheath will be inserted into the ground to a depth of 6". When a deep core is to be taken, the metal sheath will remain in the ground in order to maintain the walls of the 0-6" sample area until the next probe is positioned. Special care must be taken to prevent loss of surface soil while removing the liner from the metal probe. The resulting samples will be capped and labeled. Soil core samples may be sectioned in the field or taken to the field laboratory office for sectioning immediately after collection or after being frozen.

The 6-36" (7/8-2" OD) soil sample will be taken with a hydraulic probe by inserting the probe and contained acetate tube into the hole made by the 0-6" sampling. Extreme care should be employed to prevent contamination of the lower horizon by soil in the 0-6" zone. The soil probe and contained soil should then be inverted, the acetate liner and enclosed soil removed from the probe and **red (top)** and **black (bottom)** end caps placed on their respective ends and sections labeled (LANDIS INTERNATIONAL SOP #4.3-Current Revision, *Sampling Procedures Using Acetate/Butyrate Tubes*).

In the event that the 24-36" interval fails to fill completely, either glass wool (or similar inert material) will be packed into the liner to fill the void or the sample will be cut to size. At least 8" of soil must be present in the 24-36" interval. Samples with obvious voids within the soil column (i.e. > ½ inch) will be resampled.

At each sampling interval, a total of <number> soil cores will be collected per plot according to predetermined plot designations that are chosen randomly for sampling in each of the plots as described above. The <number> cores from each plot will eventually be composited (at the analytical lab) into one sample per depth interval prior to analysis. Core samples will be spaced representatively within each plot. Samples will not be taken from plot edges.

The <number> cores from each plot will be kept separate from those removed from other plots and designated as samples from plot "A", "B", "C", or "D" (with "A" being the non-treated plot). The column of soil for all samples should not be handled or transported in the upright position until it is sectioned into the appropriate increments. The individual increments should be capped, labeled, dated, packaged and frozen as soon as possible. It is possible that all of the samples listed above will not be collected. The Study Director may modify or terminate sampling at a particular time depending upon dissipation kinetics.

**Irrigation Water:** Prior to or at the time of the first application event and at irrigation events at approximately 3 month intervals, approximately 1L of irrigation water will be collected from the irrigation source. Additional samples may be taken if requested by the Study Director.

## SAMPLING (CONT'D)

**Spray Deposition Targets:** Spray deposition targets will be 100 mm Pyrex® Petri plates. Prior to each of the applications, Petri plate bottoms filled with 50 g of sieved soil will be placed across the nontreated plot and across each treated plot; five plates will be placed in each plot. Plates from the nontreated plot will be collected first, immediately following the respective application, followed by plates in the treated plots. Care will be taken not to contaminate the non-treated plates nor to spill any of the contents. Immediately after collection, the contents of each plate will be transferred to a separate labeled plastic bag; the corresponding empty plate will be placed in another separate plastic bag with a label identical to the corresponding soil. These samples will be frozen as soon as possible after collection and shipped frozen to the analytical laboratory. After sampling, a flag or stake will be used to mark the center of each plate location so that samples will not be collected from that location at a later date.

**Product and Water Samples:** For each field trial, <number> samples (approximately <amount> ml each) of test material, two-1 L samples of carrier water and three samples (approximately 15 ml each) of spray mix from the spray tank will be collected into appropriate containers at the time of each application. Test material and carrier water samples will be sent (ambient) to the analytical laboratory along with a photocopy of the calibration records from the GLP Field Notebook and a photocopy of the Application Verification Product/Water Sample Form via approved courier. Spray mix samples will be maintained frozen by the Field Cooperator. The Study Director may or may not elect to have these samples analyzed.

## SAMPLE SHIPMENT

**Samples Shipped from Field Site to the Analytical Laboratory:** All samples will be packed using bubble-wrap, polyurethane foam or a similar shock insulator and shipped/transferred frozen to the analytical laboratory. All packing and shipping procedures will be documented in the field logbook and a completed chain of custody form will accompany the samples during shipment. The chain of custody shall include an inventory list identifying each sample in the shipment and shall be signed and dated by the person shipping the samples. All samples will be shipped to:

ANALYTICAL LABORATORY  
<Name>  
<Address>

### **SAMPLE SHIPMENT (CONT'D)**

Packing/shipping forms provided by LANDIS INTERNATIONAL, INC., will be completed and the appropriate documents included for shipment (see LANDIS INTERNATIONAL SOP #5.12- Current Revision, *Cooperator and Laboratory Instructions for Sample Packing/Shipping Forms*).

The "**white**" and "**yellow**" copies of the packing/shipping form will be sent with the samples to the selected laboratory. Upon receipt at the laboratory, they will complete the section at the bottom of the form and retain the "**yellow**" copy for their files and return the "**white**" copy to LANDIS INTERNATIONAL, INC. The Field Study Scientist will provide the "**pink**" copy for LANDIS INTERNATIONAL, INC. archives and retain the "**gold**" copy for the GLP Field Notebook.

All samples will be packed and shipped according to LANDIS INTERNATIONAL SOP #4.14- Current Revision, *Packing and Shipping Samples*.

On the day of shipping, the Field Study Scientist (Cooperator) must contact LANDIS personnel by TELEFAX (229 242-1562), by e-mail (projectmanager@landisintl.com) or by TELEPHONE (800 526-3471), with the following information:

- (1) Protocol Number
- (2) Trial Number
- (3) Sample Numbers
- (4) Crop
- (5) Courier
- (6) Waybill Number
- (7) Date of Shipment
- (8) Destination
- (9) Condition of Samples (Frozen or Non-Frozen)
- (10) Expected Date of Arrival

### **SAMPLE ANALYSIS**

The analytical portion of the protocol will be added at a later date via protocol amendment.

### **STATISTICAL ANALYSIS**

Mean values will be calculated for summary purposes and linear regression analysis will be used to estimate dissipation kinetics. Data used for regression analyses may be transformed to achieve appropriate homogeneity of variance.

## RECORD KEEPING

Original documents or legible verified copies of the following information must be furnished to the Study Director:

- (1) A weather station will be positioned in a clear area to record temperatures and rainfall at the site. As an alternative, data will be collected from an appropriate weather station located within 20 miles (30 kilometers) of the test plots. When using this second approach, on-site weather information will be recorded on the days of application. If available, solar radiation values should be recorded at the test site or closest available location.
- (2) A description of the test site, including a map of the test plots indicating their location, topography and size, slope, and location and size of the control plots in relation to the test plots will be provided.
- (3) The soil characteristics (% sand, silt, clay, % organic matter, pH, cation exchange capacity, moisture capacity, and bulk density) of the plots (treated and nontreated) at each of the depth intervals will be provided.
- (4) Crop and pesticide use history on the plots for at least three years, and preferably five years preceding this study will be provided.
- (5) Cultural agronomic practices prior to application and during the course of the study will be provided.
- (6) The treatment date and method of application including calibration will be provided.
- (7) Climatic and edaphic conditions at each application, depth of the water table and daily soil temperatures will be provided.
- (8) Description of any post-treatment maintenance, such as use of fertilizers and pesticides, irrigation, and weeding, will be provided.
- (9) A description of the source of irrigation water and the amount will be provided.
- (10) A description of the test material (lot number, purity, and identifying codes) will be given.
- (11) The date of each sampling, description of the sampling technique, and sample handling from field to freezer will be provided.
- (12) The GLP Field Notebook will be completed and returned to LANDIS INTERNATIONAL, INC., as soon as possible after shipping the final samples of the study.
- (13) A copy of the chemical storage temperature log for the period of time that the test substance was stored.
- (14) A copy of the freezer storage temperature log for the period of time the study samples were stored.

## REPORTING

A draft report will be issued to the Sponsor upon receipt of all pertinent information. Upon approval by the Sponsor, a final report will be issued. The final report will include, but not be limited to the following:

- (1) Name and address of the facility performing the study and the dates on which the study was initiated and completed.
- (2) Objectives and procedures stated in the approved protocol, including changes in the original protocol.
- (3) Statistical methods, if any, employed for analyzing the data.
- (4) The test and control articles identified by name, code number, composition, and stability, as supplied by the Sponsor.
- (5) A description of the agricultural practices and analytical methods used.
- (6) A description of the test system.
- (7) A description of the application equipment, application procedures, and existing conditions at the time of application.
- (8) A description of all circumstances that may have affected the integrity of the study.
- (9) The name of the Study Director, other scientists and supervisory personnel involved in the study.
- (10) A description of the results to include calculation of the half-life of <test substance> with appropriate mathematical procedures.
- (11) The signed and dated reports of each of the individual scientists or other professionals involved in the study.
- (12) The locations where all samples, raw data, and the final report will be stored.
- (13) The statement prepared and signed by the Quality Assurance Unit that specifies the dates inspections were made.
- (14) The signature of the Study Director on the final report.

The final report will be transferred to the Sponsor upon study completion. Additions or corrections to the final report will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the final report that is being amended or corrected, the reasons for the additions or corrections, and will be signed and dated by the person responsible.

## **QUALITY ASSURANCE**

LANDIS INTERNATIONAL will be responsible for assuring that the study practices conform to the U. S. Environmental Protection Agency Good Laboratory Practices (GLP). This protocol will be reviewed by the Quality Assurance Unit of LANDIS INTERNATIONAL and the Sponsor. The study will be audited at critical phases and reports of findings will be submitted to the Study Director and Study Director's Management. The study report prepared by LANDIS INTERNATIONAL will be examined by the Quality Assurance Unit of LANDIS INTERNATIONAL and the Sponsor and the review will be documented by the Quality Assurance Officer's signature on the final field report.

The Quality Assurance Unit of the analytical laboratory will provide the Study Director with all facility, data, process, and report audits to meet Environmental Protection Agency GLP requirements.

**PROTOCOL SIGNATURE PAGE**

\_\_\_\_\_  
Sponsor

\_\_\_\_\_  
Date

\_\_\_\_\_  
Study Director

\_\_\_\_\_  
Date

\_\_\_\_\_  
Auditor, Quality Assurance Unit

\_\_\_\_\_  
Date