Forest Service Handbook
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Forest Service Handbook 4090.13 – Good Laboratory Practices Handbook
Chapter 60 – Protocol and Conduct of a Study

Amendment: 4090.13-93-1

Effective date: November 10, 1993

Duration: This amendment is effective until superseded or removed.

Approved by: David G. Unger, Acting Chief

Date approved:

Responsible Staff:

Last Change:

Superseded Document(s):

POSTING NOTICE. Amendments are numbered consecutively by Handbook number and calendar year. Post document in numerical order of chapters (1109.12, sec. 4.32, ex. 01). Remove entire national text of the Handbook and replace with this amendment. DO NOT REMOVE SUPPLEMENTS OR INTERIM DIRECTIVES. Retain this transmittal as the first page of this document.

Digest: Following is an explanation of the changes throughout the directive by section.

This Handbook provides direction on the principles and requirements of Good Laboratory Practice (GLPs) for Forest Service management and technical personnel. Any study that is performed with the intention of submitting the data to the U.S. Environmental Protection Agency or the U.S. Food and Drug Administration in support of a research or marketing permit must follow GLP standards.

01 - 06.5: Sets forth Authority (sec. 01), Policy (sec. 03), Responsibility (sec. 04), Definitions (sec. 05), and References (sec. 06) in the zero code chapter for the new Handbook on Good Laboratory Practices.

11 - 12.4: Establishes a new chapter that provides direction on the applicability of Good Laboratory Practices (GLPs) (sec. 11) and aspects of compliance (sec. 12).

20.3 - 22: Establishes a new chapter that provides direction on staffing requirements and training for personnel conducting, or involved with, Good Laboratory Practices.

30.3 - 35: Establishes a new chapter that provides policy and direction to determine requirements for test system care facilities (sec. 31); test system supply facilities (sec. 32); facilities for handling test, control, and reference substances (sec. 33); laboratory facilities (sec. 34); and archiving facilities (sec. 35) where Good Laboratory Practices are conducted or stored.

40.3 - 42.3: Establishes a new chapter that provides direction on equipment requirements including equipment design (sec. 41) and calibration and maintenance (sec. 42) for studies complying with Good Laboratory Practices.

50.3 - 55.33: Establishes a new chapter that provides direction on facility operations required for Good Laboratory Practices, including preparation of master schedule (sec. 51); standard operating procedures (sec. 52); reagents and solutions (sec. 53); test system care (sec. 54); and characterization, handling, and mixing with carriers of test, control, and reference substances (sec. 55).

61 - 62.32: Establishes a new chapter that provides direction on the preparation of protocols and collection of raw data for Good Laboratory Practices.

70.4 - 73.3: Establishes a new chapter, Records, Reports, and Archiving that provides direction on reporting study results (sec. 71), archiving (sec. 72).

80.1 - 81.2: Establishes a new chapter that provides direction on the requirements and duties of local and national Quality Assurance Units in support of Good Laboratory Practices.

90.1 - 93.38: Establishes a new chapter that provides direction on inspections conducted by local and national Quality Assurance Units (sec. 91 and 92) and the U.S. Environmental Protection Agency (sec. 93) in conjunction with Good Laboratory Practices.

This Handbook is now available electronically in the National Information Center in the same format as the paper copy. Henceforth, amendments to this Handbook will be issued to Forest Service units electronically on a document basis.

Table of Contents

61 - Protocols

(Sec. 01, ex. 01; 40 CFR 160.120).

61.1 - Description

Every study must follow an approved, written protocol that clearly indicates the objectives and describes all methods used in the study. The protocol is similar to a study plan (FSM 4072.3) but must include very specific information. Protocols can be sent to the U.S. Environmental Protection Agency (EPA) prior to the initiation of a study to inform EPA of the intent to perform the study and to get tentative approval of the study approach.

61.2 - Contents

A protocol must contain, but is not limited to, the following information:

1. Descriptive Title and Statement of Purpose. Give the protocol a descriptive title and state the objectives.

2. Identification of Test, Control, and Reference Substances. Include the name, Chemical Abstracts Service (CAS) number, or code number.

3. Names and Addresses. Include the names and addresses of the sponsor and the facility at which the study is being conducted. The names and addresses of independent consulting laboratories and cooperators should also be listed. Telephone numbers (including area codes) of all parties are not required in the GLP regulations but would be convenient to include.

4. Start and Termination Dates. List the following proposed dates (month, day, year) in the protocol:

    a. Experimental start date. Identify the estimated date that the test substance is first applied to the test system.

    b. Experimental termination date. Identify the estimated date on which the last data are collected directly from the study.

    These dates should be considered estimates. It is not necessary to file a protocol deviation if the experiment is delayed unless such a delay interferes with, or alters, the conduct of the study.

5. Justification for the Selection of the Test System. Some tests may follow specific standards, such as the Pesticide Assessment Guidelines of the U.S. Environmental

Protection Agency (sec. 06.5). Citing the use of such guidelines is sufficient justification. Detailed discussions of justification are needed only when the choice of test system is unusual. For example: a particular strain of a test system is used because it is resistant to nontarget insects or diseases common in the area of the test.

6. Descriptive Characteristics of the Test System. Describe the number, source of supply, species, strain, substrain, variety, age, and any other important descriptive characteristics of the test system when applicable.

7. Identification of the Test System. Describe the procedure for identifying the test system.

8. Experimental Design. Describe the experimental design, including methods for the control of bias.

9. Solvents, Emulsifiers, and Other Materials. Provide a description of the solvents, emulsifiers, and/or other materials used to solubilize or suspend the test, control, or reference substances before mixing with the carrier. The description must include specifications for acceptable levels of contaminants that should not interfere with the purpose or conduct of the study.

10. Route of Administration. Describe the route of administration and the reason for this choice. The route of administration may be mandated by specific guidelines, such as those developed by the EPA, or required by the nature of the test.

11. Dosage Level. Identify the dosage level of the test, control, or reference substance and the method and frequency of administration.

12. Diet of the Test System. Include only when animals, including insects and fish, are used in the study.

13. Tests, Analyses, and Measurements. Document the type and frequency of tests, analyses, and measurements that shall be made. The protocol should specify the samples that the researcher intends to collect, and how they are to be collected and analyzed. The exact number and types of tests or specimens may not be known at the beginning of the study; therefore, this aspect of the protocol should be written with some latitude.

14. Records. Identify the records that must be maintained.

15. Proposed Statistics. Include a statement of the proposed statistics that must be used to analyze the data.

16. Approval. Include the date the protocol was approved and signed by the sponsor and the Study Director.

A sample of a portion of a protocol that includes all of the criteria set forth in this section is presented in exhibit 01.

61.2 - Exhibit 01

Sample Study Protocol
Study Protocol

Bifenthrin: Forest Terrestrial Field Dissipation Study

Study Number BFGA910000

The University of Georgia
Cooperative Extension Service
Agricultural Services Laboratory
Athens, GA 30602

Dr. Parshall B. Bush
Study Director

April 23, 1993

TABLE OF CONTENTS

61.2 - Exhibit 01--continued

I. PURPOSE. To conduct a forest terrestrial field dissipation study to provide information on the movement and dissipation of bifenthrin when the insecticide is applied under typical use conditions to a southern pine seed orchard. The following reference was used as a guide in designing this study:

U.S. Environmental Protection Agency. December, 1989. Environmental Fate and Effects Division Standard Evaluation Procedures: Terrestrial Field Dissipation. EPA 540-0990-073. Washington, DC: U.S. Environmental Protection Agency. 29 p.

II. OBJECTIVE. The objective of this study is to determine initial residue deposition levels and residue decay patterns of bifenthrin on loblolly pine needles, forest floor litter, bare soil, and soil from the forested part of the seed orchard.

III. SPONSOR.

USDA, Forest Service
Forest Pest Management
Room 925
1720 Peachtree Road, NW
Atlanta, GA 30367
Telephone Number: 404-347-2718
Authorized sponsor representative: Dr. John W. Taylor

IV. TESTING FACILITY.

The University of Georgia
Cooperative Extension Service
Agricultural Services Laboratory
Athens, GA 30602

V. KEY PERSONNEL.

Program Coordinator:	Dr. John W. Taylor USDA, Forest Service Forest Pest Management 1720 Peachtree Road, NW Atlanta, GA 30367 Telephone Number: 404-347-2718
Study Director:	Dr. Parshall B. Bush Pesticide Chemist USDA, Cooperative Extension Service Agricultural Services Laboratory University of Georgia Athens, GA 30602 Telephone Number: 404-542-9115
Field Coordinator:	Dr. Yvette Berisford Research Coordinator USDA, Cooperative Extension Service Agricultural Services Laboratory University of Georgia Athens, GA 30602 Telephone Number: 404-542-7670
Quality Assurance Officer:	Dr. Michael E. Mispagel

61.2 - Exhibit 01--continued

University of Georgia
College of Veterinary Medicine
Athens, GA 30602
Telephone Number: 404-542-5873

VI. PROPOSED EXPERIMENTAL START DATE. May 1993.

VII. PROPOSED EXPERIMENTAL TERMINATION DATE. May 1996.

VIII. TEST SUBSTANCE.

A. Identity.

Registered Name: Capture 2EC

Active Ingredient: Bifenthrin

Chemical Name: [2-methyl(1,1'-biphenyl)-3-yl]methyl
3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate

Manufacturer: Food Machinery Corporation (FMC Corporation), Agricultural Chemical Division, 2000 Market St., Philadelphia, PA 15105

Molecular Formula: C23H22O2F3Cl

Chemical Family: Pyrethroid Pesticide

Molecular Weight: 422.88 (bifenthrin)

Appearance: Off-white to pale tan waxy solid

Odor: Very faint, slightly sweet

Melting Point: 57-64o C

Vapor Pressure: 1.81 X 10-7 millimeters mercury at 25o C

Density: 1.212 g/ml at 25o C

pH: 5.4-6.0 at 25o C

61.2 - Exhibit 01--continued

Viscosity: 18,800 centistokes at 25o C

Flash Point: 330o F by Pensky-Martens Closed Cup

Solubility in Water: Less than 0.1 parts per billion

Solubility in Other Chemicals: Slightly soluble in methylene chloride, chloroform, aceone, ether, and toluene. Slightly soluble in heptane and methanol.

Octanol/Water Partition Coefficient: >1 X 106

Storage Stability: Technical bifenthrin exhibited no loss of the active ingredient after one year at either 25o C or 50o C. Capture 2EC is stable after 3 months at 25o C and 50o C.

Mobility in Soil: Bifenthrin is tightly bound to the soil soon after application, exhibits low mobility in sandy soil, and is immobile in sandy loam, silt loam, and clay loam soils (FMC Technical Information Sheet).

Degradation in Soil: Bifenthrin is slowly degraded in soil. In laboratory studies, the half-life in soil is 65 - 125 days depending on soil type (FMC Technical Information Sheet).

Identity of the Capture 2EC Batch to be Used in the Field Tests:

Date received at the testing facility: March 18, 1992

Number and size of containers received: Three, 1-gallon metal cans

Reference number on the containers: PL 91-37

Storage conditions: The unopened drums are stored at room temperature in the testing facility's pesticide storage room.

B. Reserve Sample Deposition. A reserve sample of each batch of the following will be stored at 1-4o C in storage facilities at the Agricultural Services Laboratory:

1. Reference standard.

2. Test substance.

    a. A reserve sample of Capture 2EC concentrate will be kept from each of the pesticide containers which we use in the field tests.

b. A sample of the application solution will be taken from the airplane and hydraulic sprayer tanks on each application day.

IX. TEST SYSTEM.

A. Justification of the Test System Selection. Prior to registration of a pesticide for field use, the U.S. Environmental Protection Agency (EPA) requires terrestrial field dissipation studies on that pesticide to determine the extent of residue dissipation under actual use conditions. The orchard selected in this study is typical of southern pine seed orchards where bifenthrin would be used.

Soil for bifenthrin analysis will be sampled at 15 centimeter intervals to 90 centimeters. Bifenthrin has a very low water solubility, is essentially immobile in a variety of soils, and is relatively persistent with a soil half-life of 65-125 days. Normally soil samples for pesticides of low soil mobility and low water solubility need not be collected to 90 centimeters. However, because of bifenthrin's persistence in soil and a very small literature data base on soil mobility and degradation studies, the soil will be sampled to 90 centimeters.

Pine needles for bifenthrin analysis will be shot from trees. Trees are too tall for pole pruners and a bucket truck would add unnatural compaction to the soil.

Most of the bifenthrin which penetrates the Mead Seed Orchard canopy during application will be intercepted by the grass layer. The grass layer is very dense so comparatively less bifenthrin will reach the underlying litter. For this reason, grass and litter will be separately sampled. Each litter sample will be taken immediately below each grass sample. All of the grass within a 20 centimeter X 20 centimeter stainless steel frame will be cut level with the top of the litter layer and collected at each predetermined sampling point on each sampling day. (See the Experimental Design and Test Procedures in section X of this protocol for more details.) The litter below it will also be collected.

Use of a frame of known dimensions will enable a more concise measurement of residues per unit area in the orchard and will ensure equitability in sample size.

B. Description of the Test System. The study site is the Mead Seed Orchard located in the Georgia Piedmont, approximately 80 kilometers south of Athens, Georgia, near the town of Eatonton, Georgia (Putnam County). It is a typical pine seed orchard, planted in 1970 in loblolly pines (Pinus taeda L.) on a 7 meter X 7 meter grid (approximately 100 trees/hectare). Some trees that produced undesirable seed or were diseased have been culled so that the original 7 meter spacing is now irregular. A complete ground cover is dominated by coastal bermudagrass (Cynodon dactylon ((L.) Pers.). The orchard terrain is rolling with a maximum relief of 20 meters (66 feet) on 6-10 percent slopes. Soils are loam and clay textured Typic Hapludults of the Davidson series. Average annual precipitation is 1,400 millimeters (55 inches).

C. Procedures for the Identification of the Test System.

1. Identification of the Site. The site will be described as follows:

a. Site maps.

(1) Geographical location within the State.

(2) Geographical location within the seed orchard.

(3) Site map showing locations of all sampling sites and weather stations.

(4) Topographical map with slopes identified.

b. Soil Characteristics.

(1) The soil in the orchard will be characterized and described according to the U.S. Department of Agriculture, Soil Conservation Service classification records.

(2) Soil will also be characterized as follows:

(a) Texture (percentage of sand, silt, and clay).

(b) Percentage of Organic Matter.

(c) Moisture Content.

(d) pH.

(e) Cation Exchange Capacity.

(f) Bulk Density.

The soil analysis for the characteristics listed in paragraphs b (2) (a)-(f) will be conducted at the Soil Testing Laboratory, University of Georgia Cooperative Extension Service. The laboratory does not have a documented Good Laboratory Practices program.

(3) Soil Temperatures.

(a) Measurements. Weekly soil temperatures will be measured at the following depths: soil surface, 2.54 centimeters (1 inch), 12.70 centimeters (5 inches), 25.40 centimeters (10 inches).

(b) Locations. Soil temperatures will be measured at each of the depths listed in paragraphs (3)(a) in three locations which represent each of the following conditions:

(i) Open canopy (no overhanging vegetation).

(ii) Closed canopy (shaded forest floor).

(iii) Bare soil (outside the forested part of the orchard).

Each of these locations will be selected by visually inspecting the orchard. Because the temperature probe will be pushed into the soil and may contaminate lower soil layers with pesticide that may adhere to soil on the probe, the soil temperatures will not be measured from the areas where the soil samples are to be collected.

c. History of Pesticide Use. A history of pesticide use, over the last 5 years, in the portion of the orchard used for this study will be recorded in the study notebook.

d. Temperature and Precipitation Records. The monthly averages of temperature and precipitation over the past 10 years, or to the extent for which records are available, will be included in the study records to compare with the weather data collected during this study.

2. Identification of Samples. Because of the large variety of samples to be collected on sampling days, sample containers will be prelabelled with applicable information which identifies the sample by the following:

   a. Study Number.

   b. Collection Date.

   c. Name of the Person Who Collected the Sample.

   d. Sample Type.

   e. Sample Code.

   f. Sampling Location.

   g. Application (Aerial or Hydraulic) Site.

   Sampling information for each sample will be recorded on a chain of custody form on each collection day. Include the form number if applicable. This form will accompany samples from the field to the lab where a Pesticide Sample (PS) Number will be assigned to each separate sample. The PS Number is the number by which all samples will be followed from receipt in the laboratory to final data analysis. The PS Number will be recorded on the chain of custody form and in the laboratory log book. The laboratory log book is a daily record of all samples which are received into the laboratory for residue analysis.

X. EXPERIMENTAL DESIGN AND TEST PROCEDURES.

A. Pesticide Application. Pesticide applications will be made to simulate currently used application methods (aerial and hydraulic) for insect control on pine seed orchards. The pesticide will be applied to two sites. A site is an area to which the test substance is applied. One site is termed aerial, and the trees on this site will be sprayed from the air by airplane. The other site is termed hydraulic, and the trees on this site will be sprayed from the ground with a hydraulic sprayer. Each site will be separated from each other to preclude pesticide drift during applications. The locations of the two sites will be based on similarity of slopes, pine tree canopy, and ground cover conditions. Selection of the sites will be based on these criteria after several visual inspections of the orchard.

Three aerial applications of Capture 2EC will be made to the "aerial" site approximately 30 days apart at the rate of 0.2 pounds active ingredient/acre per application. Trees in the "hydraulic" site will be sprayed by ground hydraulic sprayer approximately 30 days apart with Capture 2EC at the rate of 0.2 pounds active ingredient/acre per application.

B. Control Samples. Control samples of soil, pine needles, forest floor grass, forest floor litter, groundwater, and forest floor leachate will be collected in each site prior to the application of the pesticide. A separate control site (an area that received no pesticide application or drift) is unavailable within the Mead Seed Orchard.

C. Irrigation. Irrigation will not be used to augment natural rainfall to reflect typical rainfall patterns. The expense and disruption of normal seed orchard activities caused by the installation and use of an irrigation system prohibit its use.

D. Forest Floor Grass, Litter, Soil, and Pine Needle Sampling. Within each forested site (aerial and hydraulic), the following samples will be collected for bifenthrin residue analysis:

1. Soil (at 15 centimeter intervals to 90 centimeters in depth).

2. Forest Floor Grass.

3. Forest Floor Litter.

4. Pine Needles.

Soil cores, forest floor grass, forest floor litter, and pine needles will be collected in randomly selected "sampling squares", described as follows.

The Mead Seed Orchard management maintains a tree location map. This map is divided into a 7 meter X 7 meter grid system and shows the original and current locations of each tree in the orchard. Each tree was planted 7 meters apart in 1970. Each tree is identified by a numbered tag attached to the tree. These tag numbers are also plotted on the orchard tree on the tree location map. For our sampling purposes, a 14 meter X 14 meter grid overlay will be superimposed on the tree location map, and each 14 meter X 14 meter area will be designated and numbered as a "sampling square."

61.2 - Exhibit 01--continued

On each sampling day, soil will be collected from depths of 15, 30, 45, 60, 75, and 90 centimeters. One sample each of forest floor grass, forest floor litter, and pine needles will be collected from each of 15 randomly chosen sampling squares. Within each sampling square, the exact location of the sampling point will be determined by a random compass direction and distance (in meters) from the center of the square. At that sampling point, grass and the underlying litter will be sampled. Soil cores will be taken immediately below the point where the litter sample was taken; needles will be sampled from the tree nearest the point at which the soil sample was taken. Samples from each of 5 sampling squares will be composited, so that on each sampling day there will be 3 subsamples of each soil core; 3 subsamples of grass; 3 subsamples of litter; and 3 subsamples of pine needles for residue analysis.

There are 36 sampling squares at each site. Each sampling square will be assigned a number prior to the experimental start date. These assigned numbers will not change during the course of the study. The Statistical Analytical System (SAS) uniform deviate function, RANUNI (SAS Institute, Cary, North Carolina), will be used to randomly select the 15 sampling squares where samples will be collected on each sampling day. The RANUNI will randomly select a compass bearing (0 - 360o), rounded to the nearest degree, and a distance, rounded to the nearest 0.1 meter, along that compass bearing, measured from the center of the sampling square where soil, grass, litter, and pine needles are to be sampled. Prior to each sampling day, the sampling points in each of the 15 sampling squares will be located and marked with surveyor's flags.

Soil, forest floor grass, forest floor litter, and pine needles will be sampled according to the schedule below. These samples will be analyzed for bifenthrin residues.

Pre-application:	Before application.
First Application:	Application day; and 3, 5, 7, 14, and 28 days post-application.
Second Application:	Application day; and 3, 5, 7, 14, and 28 days post-application.
Third Application:	Application day; 3, 5, 7, 14, and 28 days post-application; and then monthly beginning 60 days post-application and continued for 10 additional months or until the pattern of residue decline is established, whichever comes first.

61.2 - Exhibit 01--continued

Standard Operating Procedure Numbers 254-EX, 253-EX, and 255-EX provide details on sampling soil, forest floor grass and litter, and pine needles, respectively. Each sampling technique is briefly explained as follows:

1. Forest Floor Grass. Grass will be sampled from 15 sampling squares on each sampling day within each site sampled. All of the green grass above the top of the litter will be cut within a 20 cm X 20 cm stainless steel frame from 5 sampling squares. These samples will be composited in one grocery-type paper bag. A subsample will be removed from the bag, wrapped in aluminum foil, and placed in a prelabeled plastic bag with a zipper-like closure; for example: Ziploc. Grass from each of the next 5 sampling squares will be similarly sampled and composited, so that there will be 3 subsamples collected per sampling day at each site.

2. Forest Floor Litter. Litter will be sampled beneath the point where grass samples were taken. All litter above the mineral soil within the 20 centimeter X 20 centimeter square will be collected in each of 5 sampling squares and composited as described for the grass sampling in paragraph 1.

3. Soil. Soil will be sampled at 15 centimeter intervals to a depth of 90 centimeters, except on the initial application day when soil will be sampled down to 30 centimeters (since there is no chance of the pesticide moving into the soil profile at that time). The samples from each 5 of the 15 sampling squares will be composited to make one subsample/depth, resulting in 3 subsamples for each soil depth/sampling day for each site. Soil samples will be collected with a 5.08 centimeter (2-inch) diameter stainless steel hand auger. The auger bucket will be cleaned with water between sampling intervals.

4. Pine Needles. Small diameter pine branches will be shot down from the closest tree to each of the 15 points where soil and litter samples were collected. Pine needles will be composited from each of 5 trees to make three subsamples on each collection day for each site.

E. Bare Soil Plots. Soil will be sampled in a treeless area devoid of significant ground cover. This area will be designated as the "bare soil" area. Sampling in a bare soil area is necessary since the heavy ground cover in the forested part of the orchard will likely result in an insufficient amount of the pesticide reaching the soil to be available for movement through the soil profile. Sampling procedures in this area will be similar to those previously described for soil sampling in X, paragraph section D, except that the number of sampling squares may be reduced from 15, depending on the acreage available for sampling. Fifteen soil sampling points will be sampled in the bare soil area, but the 15 sampling points may be located in fewer sampling squares.

61.2 - Exhibit 01--continued

F. Weather Station. Both an electronic weather station and a backup mechanical weather station will be established in an open area of the orchard. The stations will be equipped to record daily temperature, rainfall, and relative humidity. Evaporation will be measured in a standard U.S. Weather Bureau evaporation pan and readings will be recorded from the pan at least twice weekly. The mechanical weather station will record daily readings on a 7-day strip chart in the following instruments:

1. Hygrothermograph.

2. Tipping rain gauge.

A non-recording rain gauge will also be used in the backup weather station.

G. Sample Shipment and Storage Conditions. Samples for residue analysis will be placed on ice or dry ice as soon as possible after their collection. They will then be transported to the Agricultural Services Laboratory where they will be logged in and stored at 1-4o C. Refrigerators in which samples will be stored will be equipped with an audible alarm system which records the temperature and time elapsed since the storage temperature was exceeded. A temperature log will also be maintained at each storage facility.

Soil, litter, grass, and pine needle samples will be transported and stored in the following manner:

1. Soil. Standard heavy weight soil sampling bags (paper) supplied by the Soil Testing Laboratory, University of Georgia, Cooperative Extension Service.

2. Litter. Samples will be wrapped in aluminum foil and placed in a prelabelled plastic freezer bag with a zipper closure (1 gallon capacity size).

3. Grass. Same as for litter.

4. Pine needles. Same as for litter.

H. Sample Storage Stability. A sample storage stability study will be conducted with all matrices collected for residue analysis. Each matrix will be spiked to make a known concentration of the test substance, and the samples will then be placed under the same storage conditions as the field-collected samples for a period of time at least equal to the expected storage time of the field samples. Samples will be periodically removed and analyzed for degradation of the test substance.

61.2 - Exhibit 01--continued

XI. ANALYTICAL TECHNIQUE FOR THE DETECTION OF BIFENTHRIN RESIDUES. The analytical techniques used for analyzing samples for bifenthrin residues are described in Standard Operating Procedure (SOP) No. 290-EX, Analytical Techniques for Detection of Bifenthrin Residues. Further details on the analytical technique and quality control techniques are provided in the following SOPS:

SOP Number	Topic
291-EX	Calculations used for determining residue concentrations from gas chromatography (GC) charts
292-EX	Information to be included on GC charts
293-EX	Linearity check of GC sensitivity
294-EX	Determining residue recovery levels
295-EX	Sohxlet extraction technique
296-EX	Rotary evaporation technique
2970EX	Use of gel permeation chromatography to clean up sample extracts

XII. PROTOCOL AMENDMENT. Any changes made in this protocol must have the written consent of the sponsor. Protocol amendments will be kept on file by the Study Director and the Quality Assurance Manager.

XIII. STATISTICAL ANALYSIS OF DATA. Statistical analysis of data will be conducted through Statistical Analytical System (SAS) software programs. The General Linear Model (GLM) procedure will be used to determine the significance of differences among data means.

XIV. REPORTING OF STUDY RESULTS. The following data and results will be reported in the final study report:

A. All data described in the present protocol.

B. Decay curves (if applicable) for test substance residues in soil, grass, bare soil, forest floor litter, and pine needles.

C. A description of transformations, calculations, and statistical treatment of the data.

D. A description of all circumstances that may have affected the accuracy or integrity of the data.

E. The location where all samples, raw data, and the final report are to be stored.

F. Residue half-life calculations.

G. A signed statement from the Quality Assurance Unit that specifies the date(s) that the study was inspected and that the findings were reported to management and to the Study Director.

H. Bifenthrin application information as described in SOP No. 243-EX.

I. Full description of analytical methodology from sample storage to residue analysis.

J. Discussion of leaching and persistence of bifenthrin.

K. Signatures of key personnel.

L. Good Laboratory Practice compliance statement signed by the Study Director.

XV. RECORDS TO BE MAINTAINED.

A. Study proposal and study plan submitted to and funded by The National Agricultural Pesticide Impact Assessment Program (NAPIAP) and administered through the Forest Service.

B. Study protocol and any revisions.

C. Personnel file: Resumes, training, job experience, and job descriptions for all personnel engaged in or supervising the conduct of the study.

D. Source, purchase orders, shipping labels.

E. Field data books.

F. Weather data such as daily precipitation, temperature, relative humidity, and pan evaporation.

G. Balance calibration records.

H. Pesticide Sample log which is used to log in samples and record their codes. Each sample is assigned a PS Number by which it is identified through analysis and data calculations.

I. Chain of Custody forms.

J. Instrument calibration and maintenance notebooks.

K. Refrigerator and freezer temperature records.

L. Standard Operating Procedures notebook.

M. Signature page (names, signatures, and initials of all personnel involved with the study).

N. Chromatograms of all samples analyzed.

O. Weight book of all samples weighed.

P. Quality Assurance inspections.

Q. Bifenthrin application data.

R. Site maps.

S. Soil characterization data.

T. Calculations, charts, graphs, and data analysis.

U. Residue reports.

V. Laboratory quality control records.

W. Miscellaneous study data.

XVI. STORAGE AND RETRIEVAL OF RECORDS AND DATA. Raw data, documentation, protocols, and interim and final reports will be retained in a room specifically designated for archive storage during the course of the study. The archives will be housed in a steel filing cabinet in Room 131 of the University of Georgia Riverbend Research Laboratory, 110 Riverbed Road, Athens, Georgia. The filing cabinet is not fireproof; however, the room is equipped with an overhead sprinkler system. The room also serves as a computer office used by the Study Director and Field Coordinator. Access to the room is limited; the only key to the room is assigned to the Study Director and is available to the Field Coordinator. The Field Coordinator is responsible for maintenance of the archives. A photocopy of all archival material will be kept in the Field Coordinator's office and in a building separate from the original archives. Various personnel will be assigned to handle photocopying and filing of various parts of the large body of records generated by the study. Standard Operating Procedures are established for data copying and filing.

61.2 - Exhibit 01--continued

XVII. RETENTION OF RAW DATA, RECORDS, AND SAMPLES. Field-collected samples will not be retained after Quality Assurance verification. At the conclusion of the study, all raw data, records, and archived samples of the test and reference substances will be sent to FMC for permanent archiving. Archived records shall be retained by FMC for 5 years following the signing of the final report or until the U.S. Environmental Protection Agency notifies the sponsor that these materials are no longer needed. Archived material will be available for inspection upon request by the sponsor.

XVIII. GOOD LABORATORY PRACTICES. This study will be conducted in accordance with the Good Laboratory Practice (GLP) Regulations as described in the Federal Register, volume 54, pages 34053 through 34074, August 17, 1989 (54 FR 34053-34074, Aug. 17, 1989). A statement to that effect, including a description of all deviations from GLPs, will be signed by the Study Director and included with the final report.

XIX. QUALITY ASSURANCE. In compliance with Good Laboratory Practices (GLPs), the study will be periodically reviewed by the Quality Assurance Unit (QAU) of the University of Georgia. The QAU Manager will prepare and sign a statement to be included with the final study report that will list the dates inspections were held and findings reported to the Study Director. Also included in this report will be a statement by the Study Director that the final report accurately reflects the raw data and that there were no significant deviations from GLP regulations which affected the integrity of the study. Alternatively, the Study Director will describe each deviation from the GLP regulations and how it might affect the integrity of the study.

XX. REFERENCES. Terrestrial field dissipation studies are required by Title 40, Code of Federal Regulations, section 158.290 (40 CFR 158.290) in support of registration of an end-use product intended for terrestrial use. This study protocol follows guidelines described in the following Pesticide Assessment Guideline, Subdivision N, Section 164.3:

U.S. Environmental Protection Agency, Office of Pesticide and Toxic Substances. 1989. Environmental Fate and Effects Division Standard Evaluation Procedure: Terrestrial Field Dissipation. EPA 540/09090-073. Washington, DC: U.S. Environmental Protection Agency. 29 p.

61.2 - Exhibit 01--continued

XXI. PROTOCOL APPROVAL. This protocol was reviewed and approved by the following:

Dr. John W. Taylor Sponsor Representative	Signature /s/John W. Taylor	Date 4/10/93
Dr. Parshall B. Bush Study Director	Signature /s/Parshall B. Bush	Date 4/10/93
Dr. Yvette Berisford Field Coordinator	Signature /s/Yvette Berisford	Date 4/8/93
Dr. Michael E. Mispagel Quality Assurance Officer	Signature /s/Michael E. Mispagel	Date 4/5/93

61.3 - Amendments

Document any planned changes and reasons for these changes in a protocol amendment. An amendment must be planned and cannot occur after the test substance has been applied to the test system. Ensure that these protocol amendments are signed and dated by both the Study Director and the sponsor and maintained with the protocol. Protocol amendments should be recorded and approved as soon as possible. It is not necessary to halt the conduct of the study while approval is being sought.

List changes made during, or discovered after the end of a study, as protocol deviations rather than amendments.

61.4 - Deviations

Document any deviation from the protocol. Deviations usually occur when outside circumstances prevent the protocol from being followed. Protocol deviations must explain how the conduct of the study varied from the protocol and the reason for this variation. Record such deviations as soon as possible; however, the study can continue in the interim. Deviations do not have to be approved by the sponsor but should be closely examined by the Quality Assurance Unit. List important deviations that could affect the integrity of the study in the compliance statement. Include a discussion of all deviations in the final report.

61.5 - Review and Approval

Every protocol should be thoroughly reviewed by the Quality Assurance Unit and must be approved by the Study Director and sponsor before it is signed. The study officially begins as soon as the Study Director signs the protocol. Any changes made to the protocol after it is signed must be in the form of formal protocol amendments or deviations. After the Study Director signs the protocol, the study can officially begin and the test substance can be applied to the test system.

Exhibit 01 contains a sample checklist for protocol review and exhibit 02 provides a sample concurrence format for protocol approval.

61.5 - Exhibit 01

Sample Checklist for Protocol Review
PROTOCOL REVIEW CHECKLIST

Study Number: CLTH 21402 Chlorothalonil: Field Dissipation Study

Reviewed by: John Hood (Quality Assurance Unit)

INFORMATION INCLUDED	YES	NO	NOT APPLICABLE
1. Description of study			
a. Title	X		
b. Purpose	X		
2. Identification of test substance			
a. Test substance	X		
b. Code for test substance (if any)	X		
c. Reference substance	X		
d. Control substance	X		
3. Study support			
a. Sponsor name	X		
b. Testing facility - name and address	X		
4. Dates			
a. Proposed initiation date	X		
b. Proposed termination date	X		
c. Proposed experimental start date		X	
d. Proposed experimental termination Date		X	
5. Justification for test system	X		
6. Test system identification			
a. Number	X		
b. Source of supply	X		
c. Species	X		
d. Subspecies			X
e. Cultivar			X

61.5 - Exhibit 01--continued

INFORMATION INCLUDED	YES	NO	NOT APPLICABLE
6. Test system identification			
f. Age	X		
g. Other characteristics			X
7. Procedure for identification of test system	X		
8. Experimental design			
a. Description of experimental design	X		
b. Method of control of bias	X		
9. Route of administration			
a. Description	X		
b. Reason for this choice	X		
10. Administration of test and/or control substance			
a. Dosage level (in concentration units)		X	
b. Method of administration		X	
c. Frequency of administration		X	
d. Description of vehicles used for solubilization		X	
11. Tests, analyses, and measurements			
a. Type	X		
b. Frequency	X		
12. Description of statistical methods		X	
13. Records to be maintained		X	
14. Protocol approval			
a. Date of approval by sponsor	X		
b. Signature of study director	X		

COMMENTS (Note: Responses answered "no" require an explanation):

61.5 - Exhibit 01--continued

1. Missing proposed experimental start and termination dates (fields 4c and 4d).

2. Missing description of how test/control substances administered to the test system (field 10).

3. Missing proposed statistical analysis (field 12).

4. Missing list of records that need to be maintained (field 13).

REVIEWED BY: /s/ Carolyn Boyle Date 4/10/93
Quality Assurance Unit Manager Signature

61.5 - Exhibit 02

SAMPLE CONCURRENCE FORMAT FOR PROTOCOL APPROVAL

Study Director: Jessie A. Micales

Title of Study: CLTH 21402 Chlorothalonil: Field Dissipation Study

Review of Rough Draft:

Name	Title	Draft Date	Signature	Signature Date
Jessie Micales	Study Director	4/13/93	/s/Jessie Micales	4/13/93
Carolyn Boyle	Quality Assurance Unit Manager	4/15/93	/s/Carolyn Boyle	4/18/93
Others:				
N. B. Forrest (peer review)	Soil Scientist	4/15/93	/s/N. B. Forrest	4/22/93
Approval of Final Version:				
Carolyn Boyle	Quality Assurance Unit Manager	4/28/93	/s/Carolyn Boyle	4/28/93
John Pelham	Sponsor Representative	4/30/93	/s/John Pelham	4/30/93
Jessie Micales	Study Director	5/1/93	/s/Jessie Micales	5/1/93

62 - Conducting Studies

(Sec. 01, ex. 01; 40 CFR 160.130).

62.1 - Accordance with Protocol

Conduct each study in accordance with the protocol. Thoroughly document any deviations from the protocol (sec. 61.4) as soon as the deviation is known.

62.2 - Labeling Specimens

Label any specimens collected during the study in order to identify the test system, study, type of specimen, and the date of collection. Affix this information to the specimen or the specimen container in such a way that no errors occur in identification, recording, or storage of data.

62.3 - Collecting Raw Data.

62.31 - Manual Data Collection

Record all raw data in permanent black ink, except that generated by automated data collections. Data entries must include the date and the name and position of the person making the entry. The facility should keep a list of all individuals employed in Good Laboratory Practice studies and a copy of their signatures.

Store data records in bound notebooks. Store charts, tables, instrument outputs, and data collected on separate sheets of paper in a separate binder and cross-reference it to the data notebook. Do not tape or glue these items into the data notebook since the adhesive can degrade. Sequentially number the pages of notebooks and include the study number as a header. Include a conclusion statement on the last page of the study. Cross out the remaining blank pages in the notebook after the conclusion, or write the statement "End of Study Records" on each of these pages.

Raw data can be photocopied as long as it is signed and dated by the individual making the copy and stamped with the statement "exact copy of raw data." Include a statement that gives the location of the original raw data from which the copy was made. Maintain raw data as specified in section 72. Photocopies are particularly useful for raw data that are related to multiple studies, such as weather information, field plot histories, and cold room records. Photocopy all raw data recorded on thermograph paper since it will quickly degrade.

Data corrections are permissible, but must be done by drawing a single line through the mistaken data entry in a manner that does not obscure the original entry. Sign and date all corrections and state the reason for the change. These reasons can be in the form of descriptive codes, but these codes must be clearly explained. Do not obliterate mistaken data entries with correcting fluids. Do not transcribe data unless the original entries are also available.

See exhibit 01 for a sample format of how data should be recorded.

62.31 - Exhibit 01

Sample Format for Recording Raw Data

Standardized Collection Form and Data Entry for Seed Germination Data

Protocol Number: CLTH 907039

Experiment Number: 01 – 23 Date: Time: 10 AM

Experiment Title (or Description): Effect of chlorothalonil on germination of Pinus strobus seed.

Treatment and Concentration (ug/ml)	Replication Number	No. Seeds Germinated	No. Seeds Not Germinated	Percent Germination
		a/	b/ a/	
0	1	120/118	120/0/2	98.3
				c/
0	2	117	3	95.7/97.5
0	3	115	5	95.8
		a/	a/	
1	1	120/119	0/1	100/99.2
1	2	116	4	96.7
		a/	a/	
1	3	63/72	57/48	525.5/60.0
10	1	74	46	61.6
		a/	a/	
10	2	83/82	37/38	68.3

62.31 - Exhibit 01--continued

Comments:

a/ Initial value incorrect. Detected after seeds recounted. JAM 4/10/93

b/ Initial value entered into the wrong column. JAM 4/10/93

c/ Initial value incorrectly calculated. JAM 4/26/93

Signature and Date /s/ Jessie A. Micales 4/10/93

Workbook Page No. 73

62.32 - Automated data collection

Data collected by automated collecting systems are also subject to the standards of Good Laboratory Practices. This includes any piece of instrumentation that feeds data directly into a computer. Computerized collection systems must provide the same degree of consistency and quality as manual data collection. Thoroughly test and validate all computer systems, including hardware, software, interfaces, and associated data collection devices, before they are used for data collection. The vendor should provide a supply systems development protocol which includes all applicable programming standards, test runs, and test data sets. Do not rely on the validation records provided by the vendor; make separate validation tests. Test and validate any modifications made to existing hardware and software that are being made to tailor computer applications to Forest Service needs. This may happen several years after the time of purchase. Maintain and archive all validation tests.

If the system is not totally automated, document the names of those individuals responsible for data input by date and signature at the time of data entry. The date and time of use should appear on all computer outputs. Records may be maintained as accurate reproductions, allowing media-to-media transfers, such as floppy disc to tape, while preserving raw data status.

Data collected by automated systems can be corrected if needed. However, changes in computer entries must not obscure the original data entry. It is not permissible to simply enter a file and type over a datum value. Document all corrections and include the name and the title or role of the person making the change, the date, and the reason for the change. Preserve all original data entries.