SOP Log Sheet

ID # S00184

Region: SOR

State: NC **City:** Raleigh

Location: SOR Field NC State

FRD/LRD: Roger Batts
Submitter

Effective Date: 1/29/18

Description of Material (s): 2018 SOPs

Reviewed By: Juliet Thompson 2/2/18
Sign/Date

Receive Date: 2/2/18 **Date to Archivist:** 2/2/18

File Format: E-mail ☒ **CD** ☐ **Hard Copy** ☐

Electronic copy ok to use: (Y) or No (Circle one) If no, indicated below, what needs to be done

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Date from Reviewer: 2/2/18

Date Posted: 2/5/18 **Archive Date:** 2/5/18

Archive Location: Active File Rm Cabinet 1 Drawer 4

Sign: [signature]

Comment: —

S: Facility Files- SOP Cover Sheet

9/13/16 JT

North Carolina State University is a land-grant university and a constituent institution of The University of North Carolina

Department of Horticultural Science
College of Agriculture and Life Sciences

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January 9, 2018

Dr. Liwei Gu
Food and Env. Tox. Lab.,
Dept. of Food Science and Human Nutrition
Bldg 685, IFAS, Univ. of Florida
PO Box 110720, SW 23rd Dr.
Gainesville, FL 32611-0720

Dr Gu:

Thank you for agreeing to review my 2018 field SOPs. Please find enclosed proposed SOPs for use at the NCSU IR-4 Field Research Center for 2018. All SOPs will need to be approved this year, since all have had changes. The primary change in each was that all SOPs are being submitted by only one person, me. In the past, Dr. David Monks (NCSU IR-4 Center Director) had also been included, but he and I decided that the NCSU SOPs need only be submitted by the FRD. Since every SOPs was changed, I took this opportunity to correct many grammar and syntax problems. No procedural changes were made from the existing NCSU SOPs. With approval, via your initials and date, on the front page of each of these revised SOPs, we will implement these SOP's beginning in the 2018 season. Previous versions of these revised SOPs will be retained, per SOP 1.1. Your approval is also needed on the title/signature page of the SOP packet.

I have enclosed the updated version of the NCSU SOP Table of Contents with approval dates left incomplete. After the approved SOPs are returned to me, I will insert the approval dates into the new table of contents and send to the IR-4 SOR office, so that the regional copy is complete and up to date.

Please let me know if you need any other information concerning these SOP's.

Thanks,

Roger B. Batts
Field Research Director
NC State IR-4 Center

STANDARD OPERATING PROCEDURES

FOR

MAGNITUDE OF THE RESIDUE-FIELD STUDIES

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Roger B. Batts Field Research Director	Roger B. Batts (Signature)	RBB (Initials)	1-9-18 (Date)
Liwei Gu Regional Field Coordinator or Regional Director	Liwei Gu (Signature)	LG (Initials)	1-29-2018 (Date)

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Contents - Standard Operating Procedures
2018 Season

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Contents - Standard Operating Procedures
2018 Season

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of approval

SOP#: 1.1 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Lisa Cu FB **Date:** 1-29-2018

Title: General requirements for the development, use, retirement, and retention of standard operating procedures (SOPs).

Purpose: To provide guidance to scientists conducting field trials in the development, use, retirement, and retention of SOPs for field research.

Scope: Locations conducting field trials.

Procedures:

1. Each facility where trial(s) are conducted in support of the registration of pesticides will develop SOP's for all phases of the research.

2. Generic SOPs may be provided to each facility and these SOPs will be revised to accurately reflect that facilities policies, procedures and methods. Where generic SOPs are not available, the Field Research Director (FRD) will see that the required SOPs are developed and approved prior to the use in any GLP studies.

3. The SOPs will be approved by the IR-4 Regional Field Coordinator (RFC) or other appropriate approving official. The title page should show the signature or initials of the approving official, and the date signed by the approving official. Approval may also be in the form of a dated signature on each SOP.

4. Each SOP will be reviewed annually and revised when necessary. The effective date and revision number must be changed to reflect the revision. If a particular SOP is not revised, the review will be documented by the reviewer signing the 'Reviewed by' sheet for the SOP and dating. The revision number should begin with 1 and increase sequentially with each revision. One copy of each retired SOP will be retained by the FRD and placed into the NCSU IR-4 Center archives. All other copies will be destroyed. Retained copies of SOPs will be placed in a separate file from the active SOPs so as not to be used by mistake.

5. Any deviations from the SOPs must be documented in the raw data and authorized by the Study Director (SD).

SOP: 1.1, revision 12. General requirements for the development and use of standard operating procedures (SOPs).

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 1.2 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cao **Date:** 1-29-2018

Title: Numbering system for standard operating procedures (SOPs).

Purpose: To provide a general outline for SOPs via a numbering system.

Scope: All SOPs should follow the numbering system to provide uniformity in the system.

Procedures: The numbering system for SOPs is as follows:

1. General
2. Personnel
3. Facilities
4. Equipment
5. Test System Establishment and Maintenance
6. Test Substance
7. Data Handling
8. Residue Sample Handling
9. Reporting and Retention of Data
10. Disposal of Test Substances
11. Safety and Health Procedures
12. Procedures to Handle an EPA Audit or Inspection

SOP: **1.2, revision 10.** Numbering system for standard operating procedures (SOPs).

Reviewed By:	**Date:**
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North Carolina State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 1.3 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Arrington **Date:** 1-29-2018

Title: Format for use in developing SOPs.

Purpose: To assure a uniform format in the development of SOPs.

Scope: Applies to all SOPs developed by scientists for use in the conduct of trials under GLP .

Procedures: The following is the format to be used for each standard operating procedure (SOP):

Name of Test Facility (centered)
Address (centered)
1 space
Effective Date:
1 space
SOP Number: (SOP section number as a decimal); Revision Number: (sequentially beginning with 1 for first use)
1 space
Submitted by: (Name(s) of person(s) developing the SOP); Date: (date submitted)
1 Space
Approved by: (Signature or initials of approving official); Date: (date approved)
1 Space
Title: (Title)
1 space
Purpose: (Brief description of the purpose of the SOP)
1 space
Scope: (Determines where and when the SOP is applicable)
1 space
Procedures: (Describe the operating procedures in numerical order from beginning to end so that an intelligent person with some knowledge of the process can carry out the procedures without any verbal input from other sources)
Page break

Review Sheet: (List number, revision number, and title of SOP and create table of spaces to sign and date if revision is not necessary)

Each SOP page will have document name and page number. Pages will be numbered 'page ___of X', with X being total number of pages in the SOP.

SOP: 1.3, revision 12. Format for use in developing SOP's.

Reviewed By:	**Date:**

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 1.4 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linli Gu **Date:** 1-29-2018

Title: Designation of Field Research Director (FRD) and responsibilities.

Purpose: To provide information on how the FRD is designated and outline the responsibilities of the FRD.

Scope: All test facilities where GLP trials are conducted.

Procedures:

1. The FRD is designated by the Study Director (SD) based on the recommendation of the Regional Field Coordinator (RFC) to conduct the trials. The FRD at the NCSU IR-4 Field Research Center shall be a scientist with appropriate training and experience to conduct the trials.

2. The FRD will ensure that:

   a. The trial is carried out in accordance to an approved protocol and the GLP regulations.

   b. Personnel, resources, facilities, equipment, materials and methods as necessary for the conduct of the trial are utilized.

   c. All personnel actively participating in the trials understand the trial protocols, facility SOPs, and GLP regulations.

   d. All findings reported by the Quality Assurance Unit (QAU) receive appropriate responses.

   e. All raw data, summaries and other items connected with the trials that need to be retained are archived per NCSU IR-4 Center SOP 9.5.

   f. A current copy of a master schedule for all GLP trials under his/her direction is maintained.

SOP: 1.4, revision 12. Designation of Field Research Director (FRD) and responsibilities.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 2.1 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Curtis **Date:** 1-29-2018

Title: Personnel.

Purpose: Provide information regarding personnel requirements under Good Laboratory Practices (GLPs).

Scope: All field facilities conducting trials for the registration of pesticides

Procedures:

1. The NCSU IR-4 Center will have on file current copies of a professional biography or curriculum vitae (CV), a position description, and training records for any person that collects data in GLP trials and any person that supervises trial participants.

2. The NCSU IR-4 Center will have a sufficient number of persons to carry out the trials to completion and the Field Research Director (FRD) or designee will utilize trained personnel to conduct their portion of the trials.

3. There will be a supply of safety equipment in working order and sufficiently clean to protect the health and safety of the personnel connected with the project as required by regulations, pesticide labels or the trial protocols.

4. Where the application of restricted use pesticides is required, the applicator must be certified or under the direct supervision of a certified applicator.

5. Personnel handling pesticides should be trained in accordance with the current policies and guidelines of N. C. State University.

6. Personnel documentation will be reviewed periodically and revised as needed.

7. When a person's employment with the organization ends, their training records and curriculum vitae will be archived, per NCSU IR-4 Center SOP 9.5.

SOP: 2.1, revision 12. Personnel.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 2.2 **Revision Number:** 19

Submitted by: Roger B. Batts **Date:** 1-9-18

Approved by: Linei **Date:** 1-29-2018

Title: Organizational chart, facility locations, and facility layout

Purpose: To assist in the development of an organizational chart and clarifying facility locations and layout.

Scope: All field facilities conducting trials for the registration of pesticides.

Procedures:

1. An organizational chart should describe the management structure of the institution performing the work. It should also document the reporting lines for personnel engaged in GLP studies both to the institution's management and to IR-4 testing facility management.

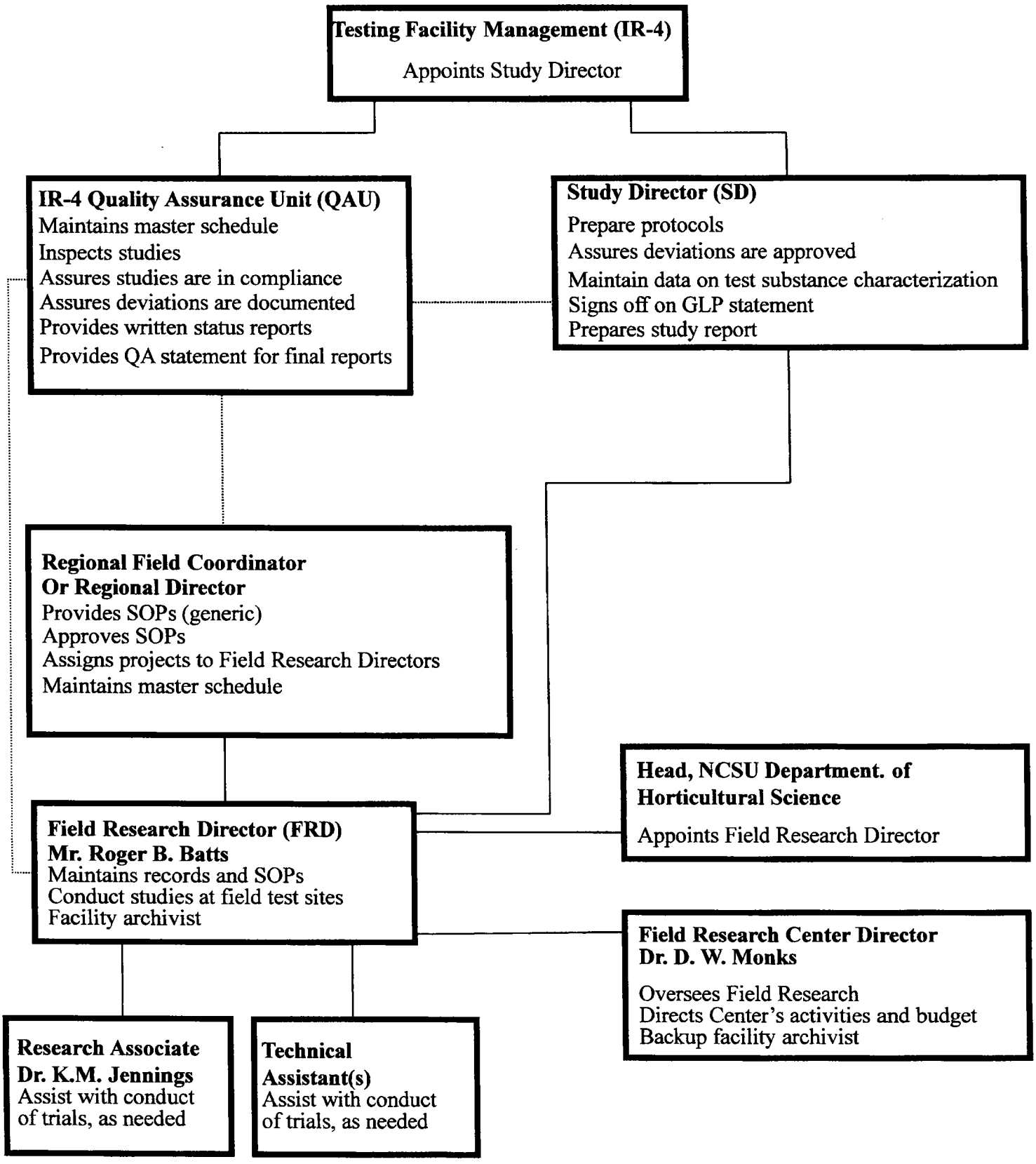
2. Each block in the chart should show the title, and a brief description of the duties of each person.

3. The head of the unit (i.e. department chair, director, etc.) should be included in the chart. This person should be the one who appoints the Field Research Director (FRD) at the institution.

4. The chart should show how the FRD and the Quality Assurance Unit (QAU) independently report to the IR-4 testing facility management.

5. Personnel engaged in the conduct of the trials should then be shown on the chart with lines of supervision, communication, and cooperation indicated.

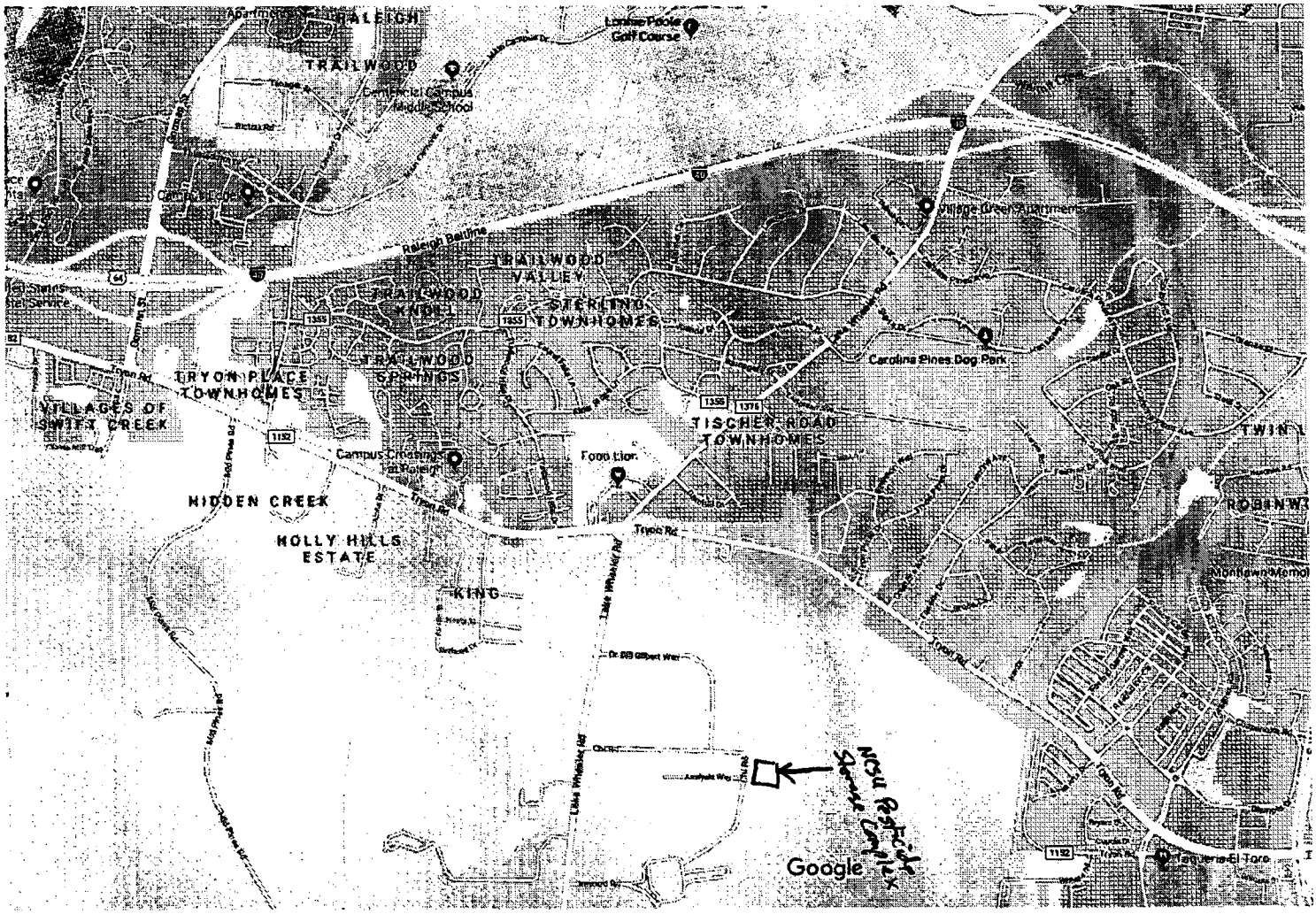
6. Maps to facilities and facility layout should be present so that an intelligent person can find equipment, supplies, and records without verbal instruction.


Testing Facility Management (IR-4)
Appoints Study Director
IR-4 Quality Assurance Unit (QAU)
Maintains master schedule
Inspects studies
Assures studies are in compliance
Assures deviations are documented
Provides written status reports
Provides QA statement for final reports
Study Director (SD)
Prepare protocols
Assures deviations are approved
Maintain data on test substance characterization
Signs off on GLP statement
Prepares study report
Regional Field Coordinator
Or Regional Director
Provides SOPs (generic)
Approves SOPs
Assigns projects to Field Research Directors
Maintains master schedule
Head, NCSU Department. of Horticultural Science
Appoints Field Research Director
Field Research Director (FRD)
Mr. Roger B. Batts
Maintains records and SOPs
Conduct studies at field test sites
Facility archivist
Field Research Center Director
Dr. D. W. Monks
Oversees Field Research
Directs Center's activities and budget
Backup facility archivist
Research Associate
Dr. K.M. Jennings
Assist with conduct of trials, as needed
Technical Assistant(s)
Assist with conduct of trials, as needed
Lines of supervision and communication
Lines of communication only

SOP 2.2 v19

Page 3 of 13

Google Maps NCSU IR-4 Center Test Substance Storage: Bldg 6A, 4021 Chi Rd., Raleigh, NC. I-40 to Lake Wheeler Rd (Exit 297). South to Chi Rd. Turn left. Con 6 inside fenced area on left.



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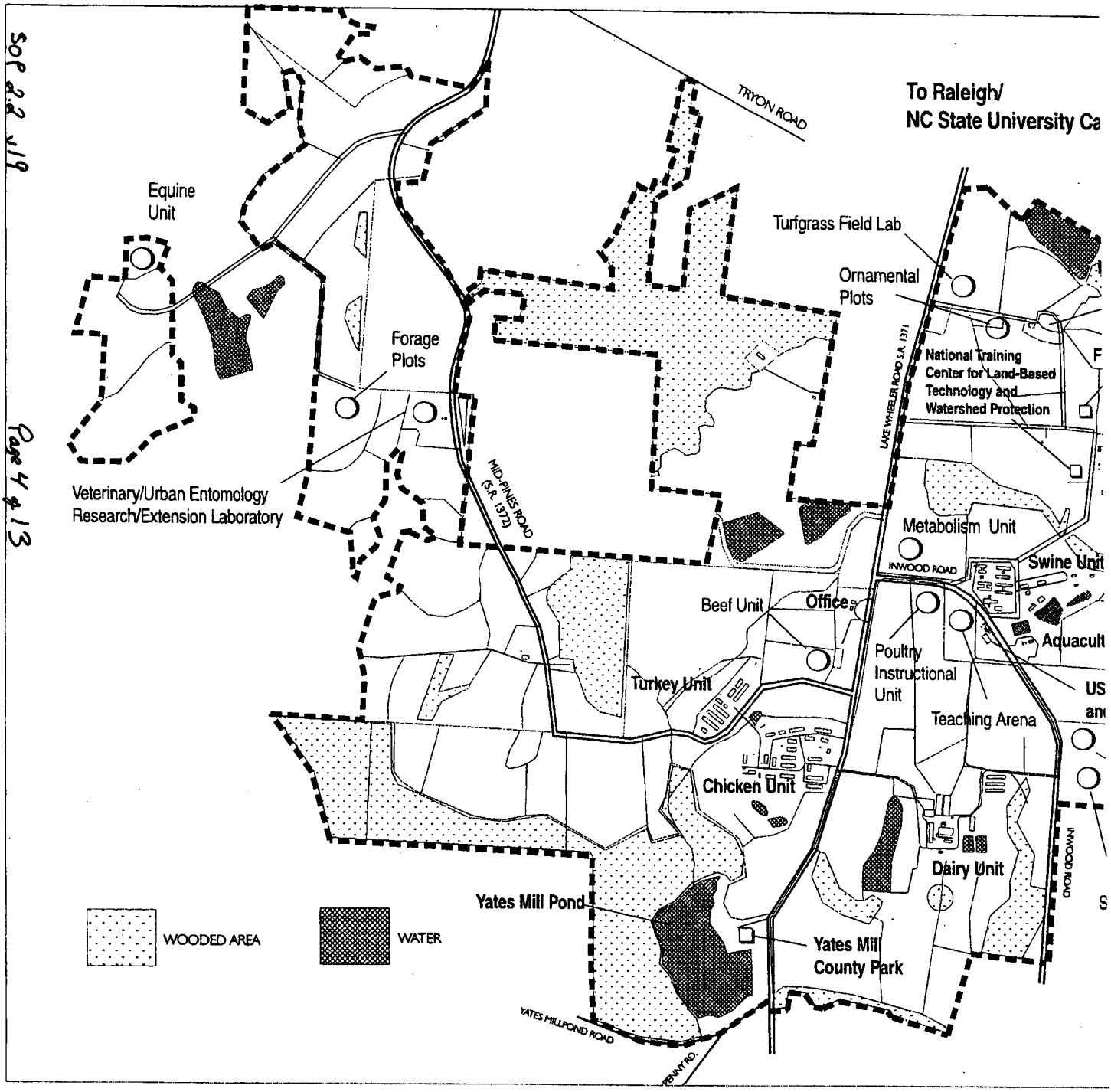

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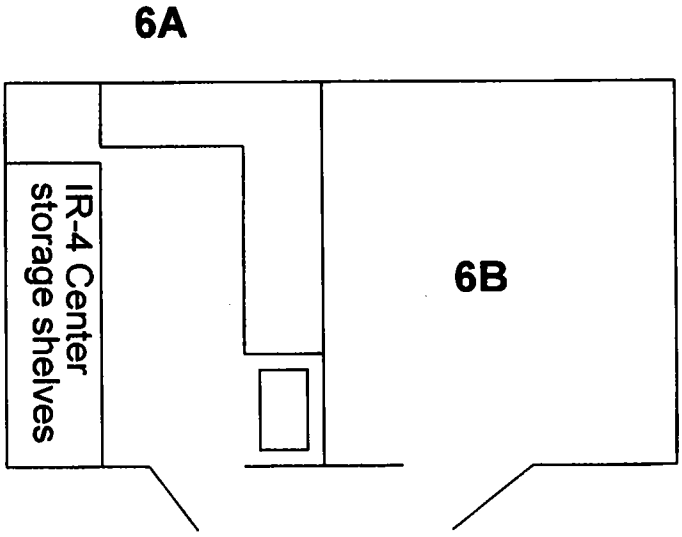
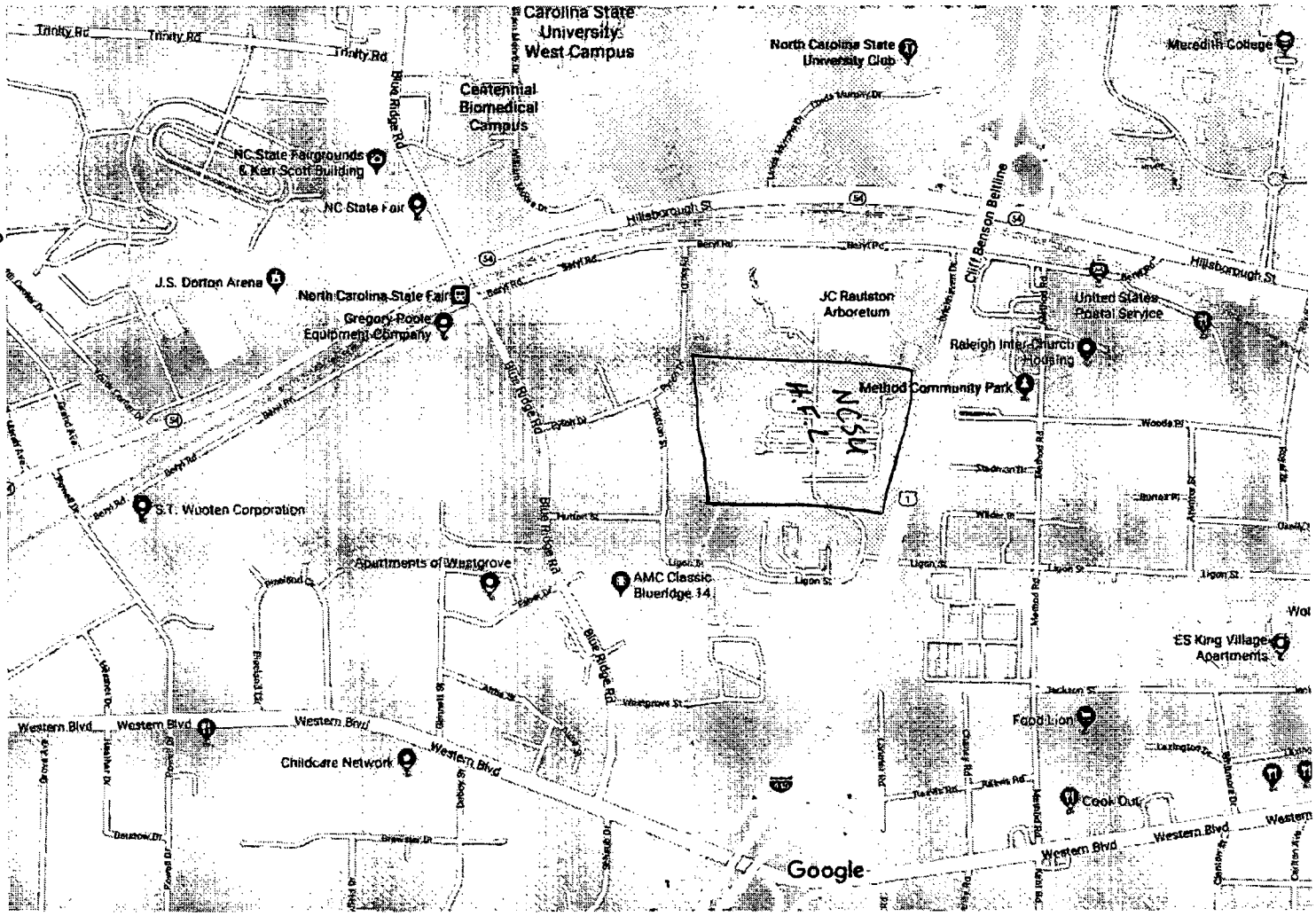
NC State IR-4 Field Research Center
Test Substance Storage

Building 6A, Lake Wheeler Field Lab., 4021 Chi Rd., Raleigh, NC



Date: January 20, 2011

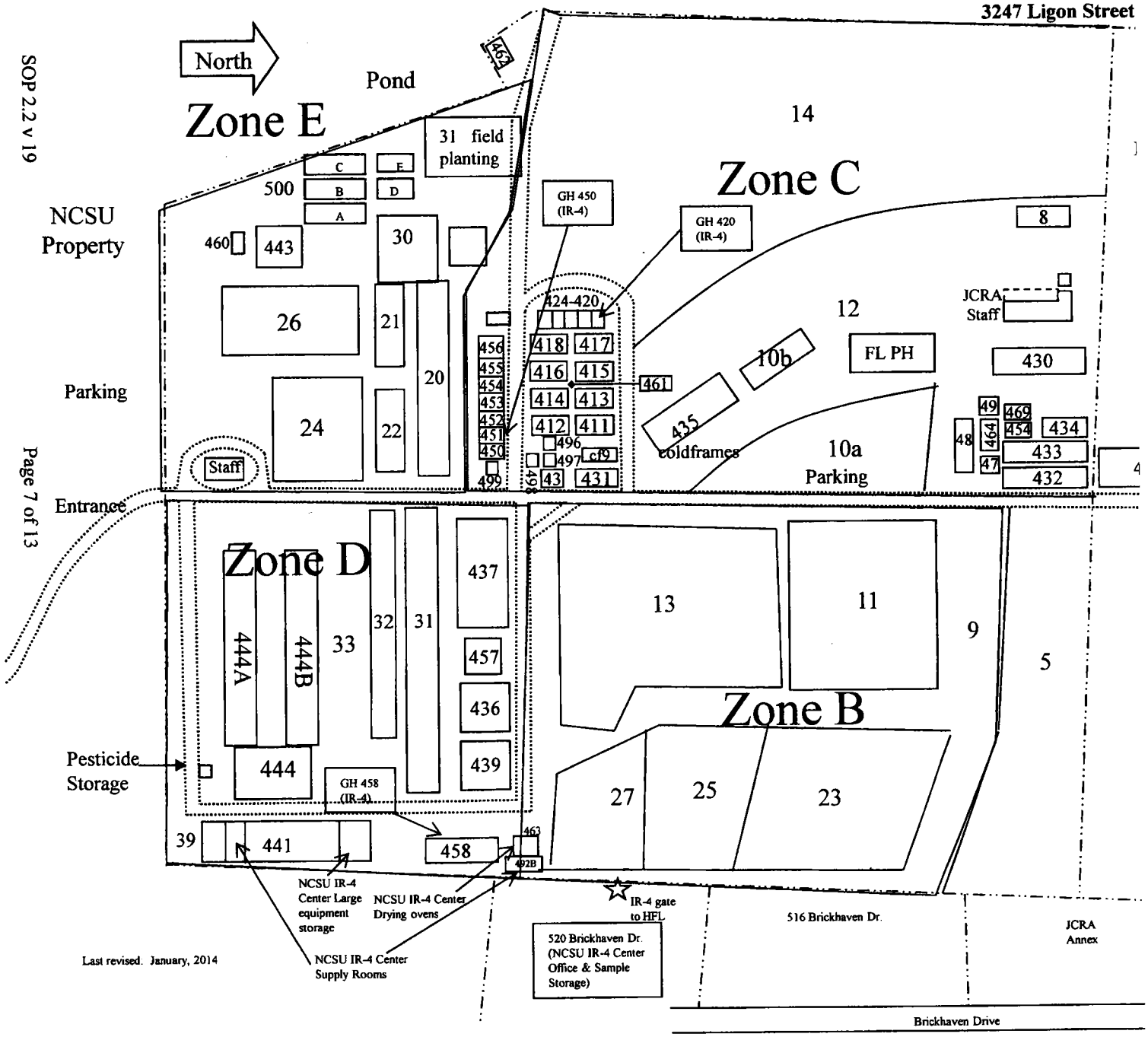
Google Maps NCSU Hort. Field Lab. 3247 Ligon Street, Raleigh, NC. I-440 to Hillsborough St. (Exit 3). East to Beryl Rd. Turn right. Continue to Method Rd. Turn l continue through tunnel under I-440. Turn right onto gravel road to HFL entrance



SOP 22 v19

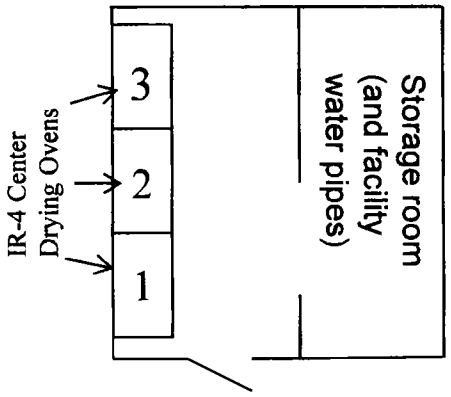
Page 6 of 13

Horticultural Field Lal
3247 Ligon Street



NC State IR-4 Field Research Center Drying Facility (Bldg. 463)

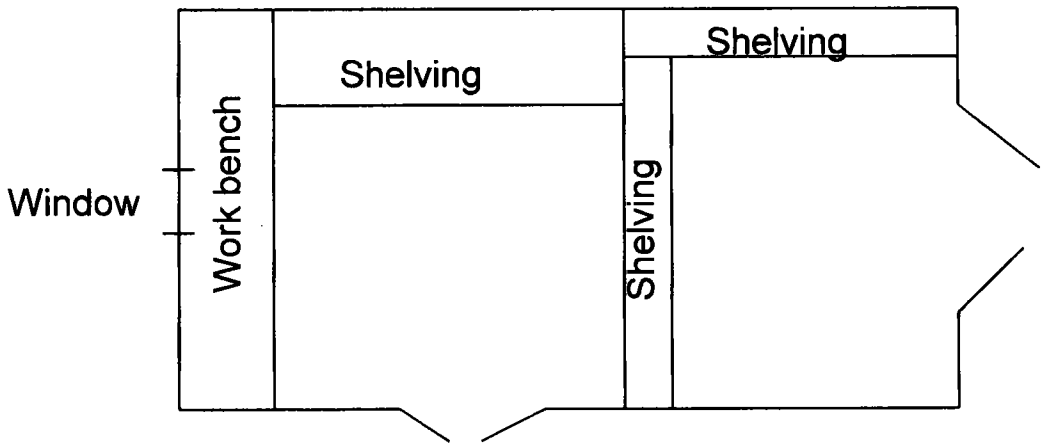
Horticultural Field Lab., Ligon St. extension., N Campus



Date: December 7, 2012

NC State IR-4 Field Research Center Supply I (Bldg. 492B)

Horticultural Field Lab., Ligon St. extension., I Campus

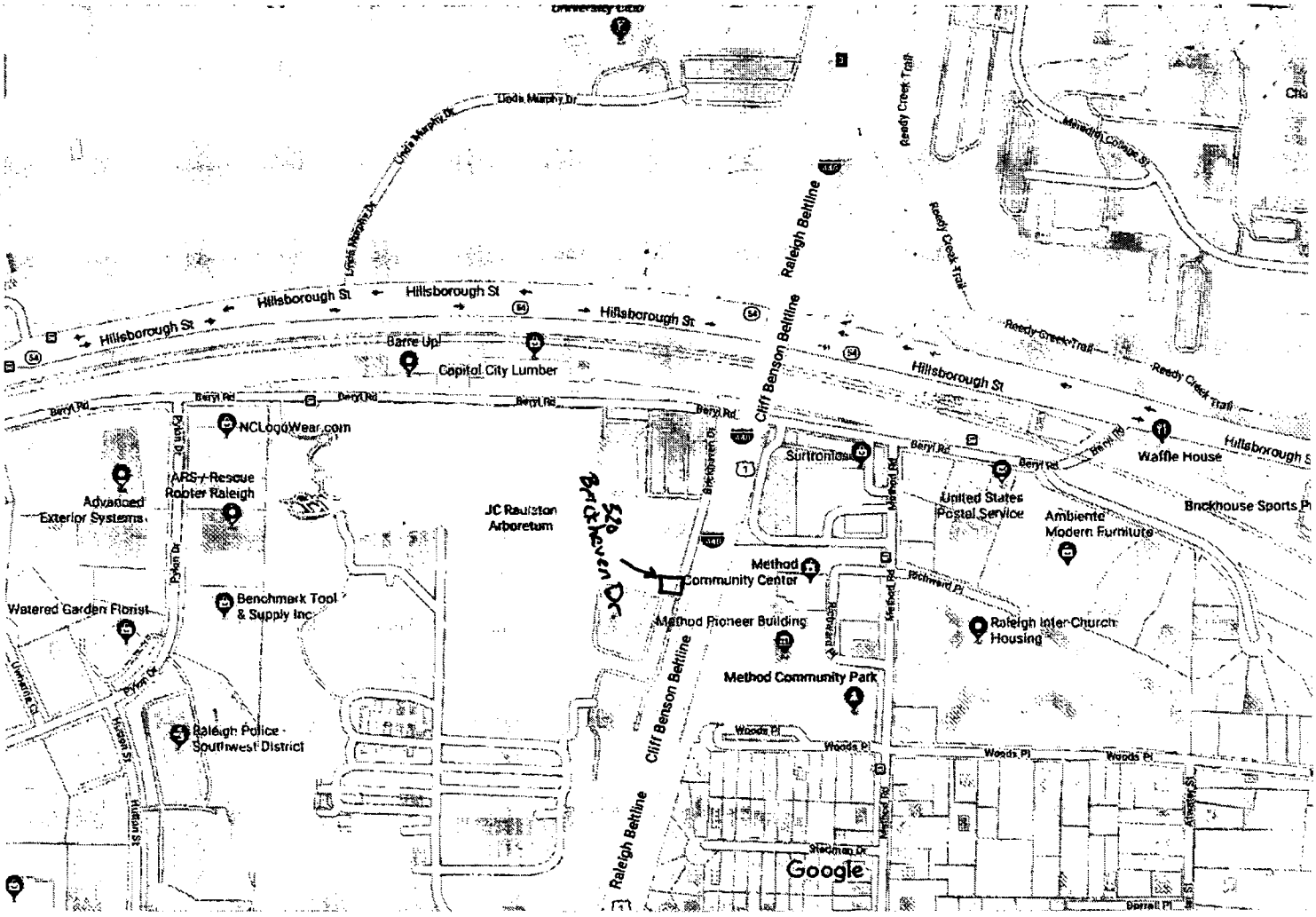


Date: December 7, 2012

SOP 2.2 v19

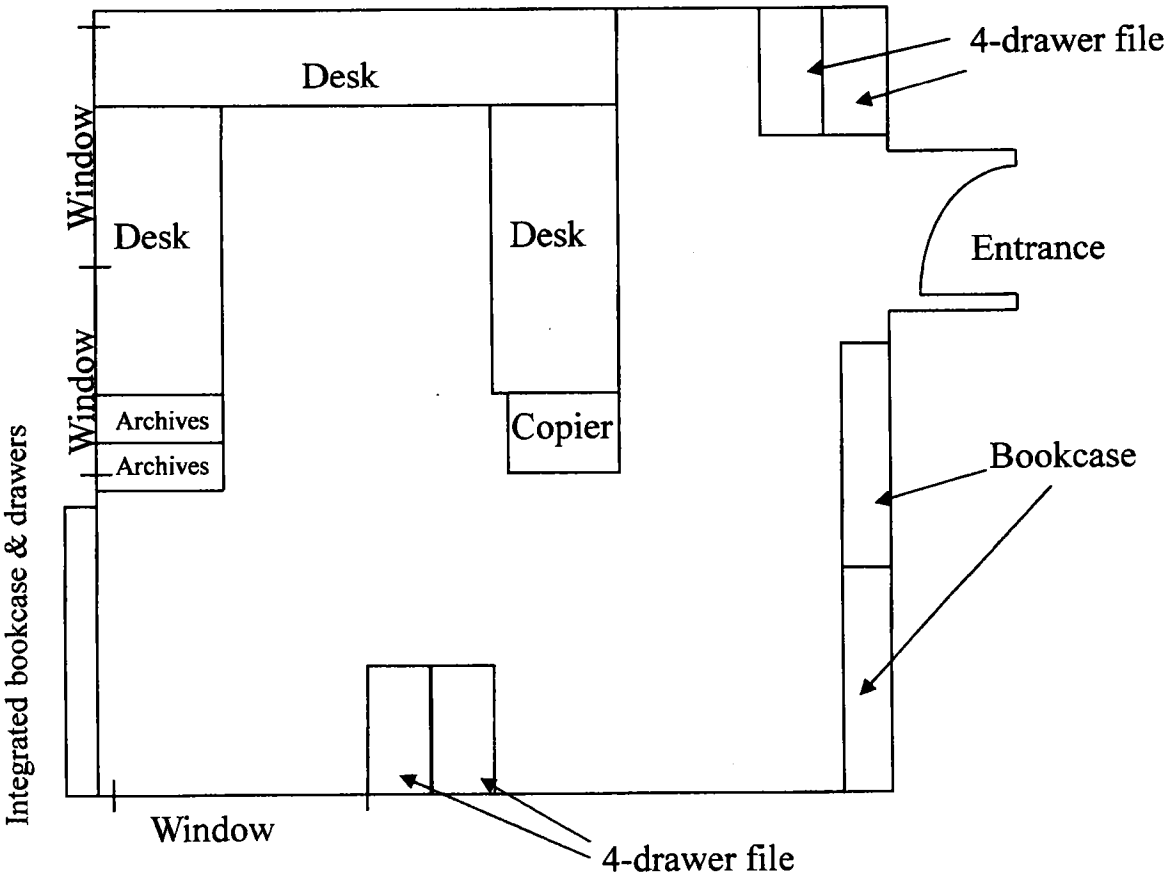
Page 10 of 13

Google Maps NCSU IR-4 Center Office and Sample Storage. 502 Brickhaven Drive, Raleigh, NC. I-440 to Hillsborough St. (Exit 3). East to Beryl Rd. Turn right. Co onto Brickhaven Drive. Continue to 520 Brickhaven Dr. at end of road.



NC State IR-4 Field Research Center Offic

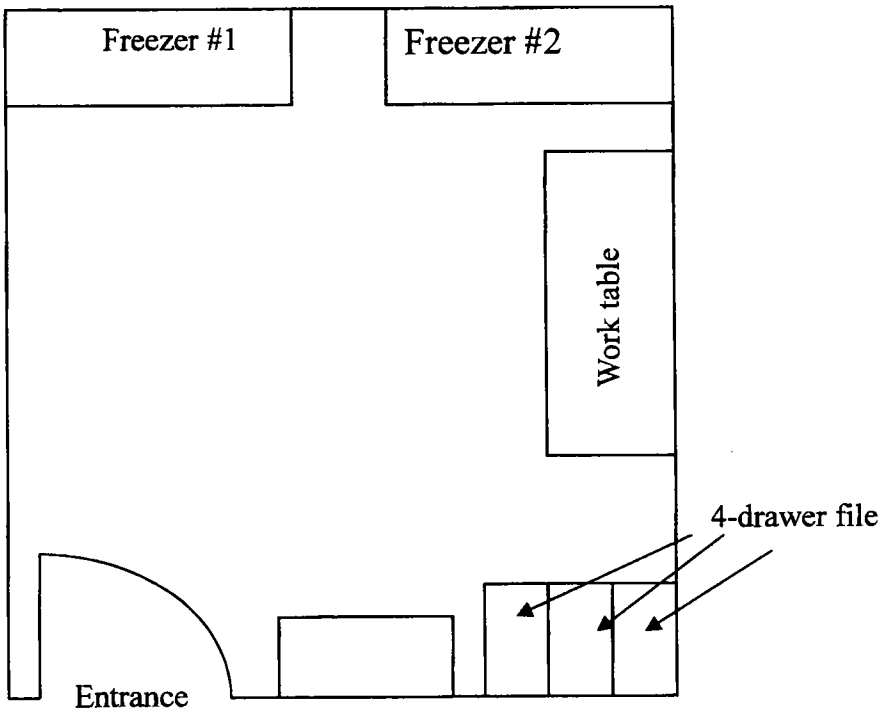
Room 110, 520 Brickhaven Dr., Raleigh, NC



Date: January 3, 2017

NC State IR-4 Field Research Center Sto

Room 111, 520 Brickhaven Dr., Raleigh,



Storage Cabinet

Date: January 20, 2011

SOP: 2.2, revision 19. Organizational chart, facility locations, and facility layout

Reviewed By:	**Date:**
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N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 2.3 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei Gu **Date:** 1-29-2018

Title: Documentation of training.

Purpose: To assure that training for personnel involved in GLP trials is properly documented.

Scope: All field facilities conducting trial(s) for the registration of test substances.

Procedures:

1. All training of personnel engaged in GLP trials should be documented in a training record, to be kept at the field facility.

2. Training received from any source, should be noted as to the name of the event, date(s) of attendance, instructor(s) name(s), and subject(s) covered. A copy of any type of certificates issued should be retained in the personnel files at the location.

3. Training on specific procedures and/or standard operating procedures (SOPs) should also be documented. Record the name of the person giving the instruction, the name of the person receiving the instruction, the date given and a concise statement of the instruction, or SOP (e.g. Dr. X explained to Mr. Y how to label the sample bags as per SOP xx on 6/2/18).

4. Each person that collects data or supervises participants in GLP trials should have read and understood those sections of the protocol and the SOPs that pertain to their responsibilities. The Field Research Director (FRD) should record in their respective training records, the names of the personnel and dates that the SOPs were explained to them. This information should be placed in the personnel file.

SOP: **2.3, revision 10.** Documentation of training.

Reviewed By:	**Date:**

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 3.1 **Revision Number:** 16

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linli Cui **Date:** 1-29-2018

Title: Guidelines for test substance and adjuvant storage and labeling.

Purpose: To assure that all test substances and adjuvants that are used in GLP trials are stored in a manner consistent with GLP requirements.

Scope: Locations conducting IR-4 field trials and where institutional guidelines do not exist for storage of test substances and adjuvants that are used in GLP trials is required.

Procedures:

1. Test substances and adjuvants that are used in GLP trials will be stored in a dry, well-ventilated building which is separate from offices, laboratories and sample storage areas. This area area should be sufficient to allow storage of the products according to their label directions. Products will be stored in accordance with current policies and guidelines of N. C. State University.

2. The temperature range within the storage facility will be monitored by a thermograph and a minimum/maximum thermometer. Data from both will be collected and/or recorded approximately monthly. The minimum/maximum thermometer will serve as a backup to the thermograph in the event it fails.

3. The original containers for all GLP test substances must be returned to the manufacturer or retained until completion of the study and/or permission is given by the Study Director (SD) to dispose of the containers. More guidance on IR-4 test substance container disposal can be found at:

   http://ir4.rutgers.edu/Other/Advisories/advisory2005-01.pdf

   Researchers may also visit

   http://ir4.rutgers.edu/FoodUse/Food_UseSimple3.cfm

   to obtain a listing of containers that he/she may discard.

4. The storage facility should have limited access by utilization of a lock and key so that only authorized persons may have access to GLP test substances.

5. Items listed below will be the responsibility of University officials overseeing the pesticide storage complex where the test substances are stored:

   a. Place highly visible, waterproof identification signs in and around the pesticide storage complex to advise of the hazardous nature of the storage facility's contents.

   b. Make accessible, materials such as adsorptive clay, granulated activated charcoal, hydrated lime, and etc. for emergency treatment detoxification of spills or leaks.

6. Do not keep products on the floor or the storage area.

7. Storage areas and mixing areas should be separate to prevent potential contamination or mixup.

8. Test substance containers shall have the following information present on the label or container surface:

   a. Name
   b. Batch or lot number
   c. Expiration date
   d. Storage conditions specific to the test substance
   e. Container sequence number of the container (i.e., 1 of *X*, 2 of *X*, etc.)

9. Adjuvants used in GLP trials will have the following listed on the container label:

   a. Name
   b. Concentration
   c. Storage conditions
   d. Expiration date

   If adjuvants that are used in GLP trials do not have a known expiration date at the time of receipt, an expiration date will be assigned by NCSU IR-4 personnel. This date will be no more than 5 years from the date of receipt.

10. The integrity of test substances and adjuvants and the prevention of product contamination will be of paramount concern at the NCSU IR-4 Field Research Center. Practices and procedures related to these issues will include, but not be limited to, the following:

a. The physical properties of the test substance and adjuvant (i.e., color consistency, odor, etc.) will be examined at each use. If any attribute is questionable, the product will not be used. If a test substance is deemed unusable, the SD will be immediately notified for guidance. If an adjuvant is deemed unusable, an appropriate adjuvant may be substituted for use. This substitution will be thoroughly documented in the Field Data Book (FDB) and/or appropriate log(s).

b. Test substance and adjuvant integrity will be further protected by controlling the temperature range to which it is exposed. This will be done at the NCSU IR-4 Field Research Center by transporting the product to the test site inside a cooler, using blue ice to avoid excessive temperatures. Since adjuvants often arrive in containers too large to be placed in such a cooler, the NCSU IR-4 Field Research Center will use smaller secondary containers of the adjuvant that will fit into the same cooler as the test substances, thus allowing exact temperature monitoring during transit and use in the field. Secondary containers must be labeled with the same information required on the original adjuvant container (See #9 above).

c. When measuring the calculated amount of test substance or adjuvant for a spray mix, the pipette or syringe used to extract the desired amount will not have been previously used and will not be used for any other product.

d. The pipette or syringe used to extract a desired amount of a test substance or adjuvant will only be used once, with the exception of multiple aliquots of 60 ml syringe described in SOP 4.2.

e. If it becomes necessary to use a temporary container for some of the adjuvant (i.e., beaker, sterile cup, etc.), any product remaining in the temporary container after the measured amount is withdrawn will be discarded. It will not be returned to the original, or appropriately labeled secondary, container.

SOP: 3.1, revision 16. Guidelines for test substance and adjuvant storage and labeling.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 3.2 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Au **Date:** 1-29-2018

Title: Site selection for field trials.

Purpose: To help ensure plots are large enough to obtain the required data or samples with sufficient uniformity and can be relocated after the trials are terminated.

Scope: Locations conducting field trials.

Procedures:

1. Site selection will be made in accordance with the horticultural practices acceptable for the commodity and the requirements established by the protocol.

2. Site will be large enough to accommodate the required number of replicates, buffer zones and treatments in accordance with an approved study protocol. It should also be large enough for the commodity to be grown under simulated commercial conditions yielding samples of sufficient size to comply with protocol sample size requirements.

3. Locate site with sufficient isolation to prevent contamination of the test plots by spray drift sources such as commercial operations or other research trials.

4. Where samples for residue trials are required, locate nontreated plots within the same area (preferably upwind and up slope of the treated plot(s)) but with enough isolation to produce nontreated, noncontaminated samples.

5. If the commodity is not required to be newly established, select a site that has commercial standards for production.

6. Prepare a plot map containing all required items from protocol and Field Data Book (FDB) instructions. Include this map in the FDB.

7. Label each plot with the field ID number and treatment as a minimum. If statistical analysis is to be performed on the data, assign the replicates and treatments to the plot map using a commonly accepted statistical design with

sufficient information to identify the replicate and treatment assigned to each plot

8. Identify both ends of each plot with a marker of sufficient visibility to be seen easily throughout trial duration.

SOP: **3.2, revision 12.** Site selection for field trial(s).

Reviewed By:	**Date:**

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 3.3 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cu **Date:** 1-29-2018

Title: Greenhouse/shadehouse facilities.

Purpose: To assure that greenhouse/shadehouse facilities are properly maintained and in sufficient working order throughout the trials to obtain data useful in the registration of pesticides under GLP guidelines.

Scope: All locations where greenhouse/shadehouse trials are performed.

Procedures:

1. Lighting, temperature, humidity, and shade levels should be sufficiently uniform in the greenhouse/shadehouse to provide nearly uniform plant growth throughout the trial area. If, due to size of trial or structure(s), a trial requires more than one structure, lighting, temperature, humidity, and shade levels should be sufficiently uniform across each to provide nearly uniform plant growth throughout the trial. Plots will be identified as described in SOP 3.2.

2. The walls, floors, and ceilings of the greenhouse/shadehouse should be maintained in good condition. Floors, benches and isles should be free of debris, weeds and superfluous equipment and should be well-drained to prevent the buildup of excess moisture.

3. Sufficient monitoring devices should be in place, in good working order, and calibrated to assure that the proper environmental conditions are maintained throughout the trials. Calibrations and maintenance activities for GLP-maintained equipment will be documented in the maintenance logs for these items.

4. Where more than one trial is conducted in a greenhouse/shadehouse, there must be sufficient isolation between the trials to prevent contamination or interference between trials.

5. Greenhouses should be equipped so as to maintain environmental conditions to simulate commercial greenhouse production techniques or as required by the study protocol.

6. Document cultural practices used in the greenhouse/shadehouse in the raw data notebook and/or greenhouse/shadehouse logbook(s).

SOP: 3.3, revision 13. Greeenhouse/shadehouse facilities.

Reviewed By:	Date:

N. C. State University, IR-4 Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.1 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Corbett **Date:** 1-29-2018

Title: Calibration and use of balances.

Purpose: To assure an accurate weighing of dry test substances.

Scope: All field facilities where a dry material is weighed for use in a field, greenhouse or hothouse trials.

Procedures: The methods, materials, and schedules for routine inspection, cleaning and calibration will be:

1. Standard weights will be calibrated/standardized every two years or less by the N. C. Department of Agriculture and Consumer Services, Division of Standards and Weights. Documentation of calibration/standardization will be kept on file.

2. Prior to each use, the user will visually inspect the balance for cleanliness. Any dirt or chemicals within the chamber or on the pan must be cleaned immediately.

3. Prior to each us, balance accuracy checks should be performed, using two standard weights that bracket (in the weight range of the chemical samples being weighed) the amount to be weighed. Record declared weights and actual weights of standards as raw data.

    a. If the measured weights of both standard weights are within 2% of the standard weights, proceed with weighing.

    b. If the measured weight of either standard weight differs by more than $\pm$ 2% from the standard weight, recalibrate the balance.

    c. If, after recalibration, the measured weights of both standard weights are within $\pm$ 2% of the standard weights, proceed with weighing and record the measured weights.

    d. If, after recalibration, the measured weight of either standard weight still

differs by more than ± 2%, replace the defective weight. If it is determined that the problem is the balance, it should be serviced before further use.

e. If desired test substance amount is less than lowest certified weight available, the two lowest certified weights will be used to ensure balance is working accurately

4. Select an appropriately-sized vessel to hold the desired amount of test substance and tare it on the scale following the manufacturer's directions in the appropriate technical manual.

5. If taring the vessel is not practical, record the weight of the vessel, add the weight of the desired amount of test substance to it and weigh out this amount.

6. Select and use appropriate safety equipment while handling the test substance.

7. Any interim container used for the test substance should be adequately labeled to prevent possible confusion at mixing.

8. Remedial actions to be taken in case of failure or malfunction include:

   a. Any problem should be immediately reported to the Field Research Director (FRD), documented, and placed in the balance records as non-routine procedures.

   b. If the problem cannot be corrected by instructions from the manufacturer's manual, a service representative should be notified. All corrective actions taken shall be documented in the balance records as non-routine procedures.

9. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

SOP: 4.1, revision 13. Calibration and use of balances.

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N.C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.2 **Revision Number:** 14

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linel Cox **Date:** 1-29-2018

Title: Measuring liquid formulations.

Purpose: To assure an accurate measurement of liquid test substances.

Scope: All field facilities conducting field trials for IR-4.

Procedures:

1. Obtain a clean cylinder or measuring device large enough to hold the volume of liquid needed, graduated in increments small enough to read to an accuracy within +/- 2% of the total volume required (i.e. if 100 ml is needed the smallest division on the cylinder should be, 2 ml or less). The following devices will be used to measure the following known volumes:

Volume to be Measured	Device to Use
1 to 5 ml	5 ml disposable syringe
5 to10 ml	10 ml disposable syringe
10 to 30 ml	20 ml or 30 ml disposable syringe
30 to 60 ml	60 ml disposable syringe
>60 ml	multiple aliquots of 60 ml disposable syringe or graduated cylinder

2. If the opening of the graduated cylinder is too restrictive to allow filling without danger of spillage, do one of the following:

   a. Use a clean container with a pour lip as an intermediate and fill the cylinder/device from it

   or

   b. Use a clean funnel that is large enough to allow filling the cylinder with a minimum of spillage.

3. If mouth of container is too restrictive to allow access of syringe, use a clean container as an intermediate from which to draw the aliquot with a syringe.

After use, return unused amounts to original container.

4. Select and use appropriate safety equipment while measuring liquids.

5. When amounts >60 ml are required, measurement with a graduated cylinder is permitted. Place the graduated cylinder on a level surface and take the reading of the liquid in the graduated cylinder at the bottom of the meniscus with the eye being level with the bottom of the meniscus. Document the amount of test substance measured in the raw data book.

6. Any graduated cylinders used to measure or transfer the test substance concentrate, as described in #5 above, should be triple rinsed and then thoroughly washed with water plus soap (or ammonia) after use to ensure that they are clean and cross-contamination of pesticides will not occur. Finally, rinse thoroughly to remove soap (or ammonia) from cylinder.

SOP: 4.2, revision 14. Measuring liquid formulations.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.3 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Jinei CB **Date:** 1-29-2018

Title: Calibration and cleaning of a liquid sprayer.

Purpose: To determine the delivery rate of a liquid sprayer and make adjustments as necessary to ensure an accurate application of the test substance.

Scope: All facilities where a liquid sprayer is used in the application of test substances.

Procedures: The methods, materials, and schedules for routine inspection and calibration should include:

1. Always calibrate before initial test substance application. For multiple applications, recheck the calibration to confirm accurate delivery (e.g., ± 5% of initial calibration or as specified by the protocol).

2. Prior to use, visually inspect pumps, hoses, pipes, fittings, regulators, pressure gages, and tanks for obvious wear or potential leaks and repair or replace as necessary as part of routine maintenance. Record any maintenance performed in the appropriate log(s).

3. Refer to the protocol for any specified application requirements.

4. Gallons per minute (GPM) will be calculated by collecting output, in milliliters (ml) from each nozzle for 15 seconds; multiplying this value by four and then dividing by 3785. Output from each nozzle will not vary by more than 5% from highest to lowest. Any part(s) contributing to values outside this range will be cleaned or replaced. This procedure will be repeated until output of all nozzles fall within required parameters.

5. Once application speed, in miles per hour (MPH), nozzle spacing (NS), in inches, and sprayer output, in GPM, are known, gallons per acre (GPA) can be calculated with the following formula:

$$GPA = \frac{GPM * 5940}{MPH * NS}$$

6. Speed will be determined by timing (sec) the movement of the sprayer over a known distance (D) or by selecting a speed, calculating the needed pass time, and adjusting until this pass time is matched. This can be done by using the following formula:

$$MPH = \frac{(D/sec)}{1.47}$$

7. Operator must carefully operate equipment, during application, under the same conditions as during calibration. Ensure solution is thoroughly mixed before application and continue to agitate during application if possible. The test substance must be applied uniformly to the entire test area.

8. After the application, the container/tank will be triple-rinsed with water, then rinsed with ammonia, then triple-rinsed with water again. Spray system will be flushed through with an ammonia/water mixture, then flushed through with water alone. Cleaning should be recorded in the appropriate log(s).

9. Remedial action to be taken in case of failure or malfunction should include:

    a. Immediately report malfunction to the Field Research Director (FRD).

    b. If problems occur during application, refer to SOP 6.5.

    c. Any repairs and replacement of parts, other than changing nozzles and/or strainers while creating a different setup of the boom, will be documented as non-routine maintenance in the appropriate maintenance log(s).

    d. Changes of nozzles and/or strainers while creating a different setup of the boom will be documented as routine maintenance in the appropriate log(s)

10. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

11. When not in use, equipment used for GLP trials will be kept in a secure location so as to avoid the possibility of contamination and tampering.

This may include a locked cover on the bed of a truck.

SOP: 4.3, revision 13. Calibration of a liquid sprayer.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 4.4 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linel CPO **Date:** 1-29-2018

Title: Calibration and use of granular applicators.

Purpose: To determine the delivery rate of the granular applicator and make adjustments as necessary to ensure an accurate application of the test substance

Scope: All facilities where a granular applicator is used in the application of test substances.

Procedures: The methods, materials, and schedules for routine inspection and calibration should include:

1. Prior to use, determine that the applicator is in good working order and mechanical condition. Make sure that the openings to release the granular material are not clogged and are free of debris.

2. Ground driven applicators:

   Following protocol requirements, perform calculations to determine actual pounds (or appropriate measures) of materials per acre (or appropriate measure).

   a. Measure an area of 0.01 acre or 435.6 square feet in close proximity to the area to be treated. A simple method to calculate the distance is:

$$\text{feet to travel} = \frac{435.6}{\text{(application width in feet)}}$$

   b. Approximate setting of the openings to operate the applicator for the desired amount of active ingredient/acre.

   c. Wear protective clothing as required by product label and fill the spreader with enough material to ensure proper operation. Operate the applicator over the measured distance and collect the output.

d. Weigh the material collected and multiply by 100 to give the amount applied per acre.

e. Repeat steps "a" to "d" until the desired rate is achieved within 5% of the total/acre

All calibration data and calculations should be recorded in the raw data

3. Non-ground driven applicators.

Following protocol requirements, perform calculations to determine actual pounds (or appropriate measures) of material per acre (or appropriate measure).

a. Measure an area of 0.01 acre or 435.6 square feet in close proximity to the area to be treated. A simple method to calculate the distance is:

$$\text{feet to travel} = \frac{435.6}{\text{(application width in feet)}}$$

b. Determine how long it will take the applicator to travel that distance.

c. Determine the amount of material needed to treat 0.01 acre and the approximate setting of the openings to operate the applicator for the desired amount of active ingredient/acre.

d. Turn the applicator on and operate it for the time required to travel the distance determined above while collecting the output from the applicator.

e. Weigh the collected material from the applicator and multiply by 100 to give the amount applied per acre.

f. Continue to change settings of the openings on the applicator until the desired rate is achieved within 5% of the total/acre.

4. Applicators must carefully operate under the same conditions as during calibration. All discharge openings should be unobstructed and flow from each should be identical. The test substance must be applied uniformly to the entire test area.

5. Thorough cleaning of the applicator will be done after each period of use

and when changing test substances.

6. Remedial action to be taken in case of failure or malfunction should include:

   a. Immediately report malfunction to the Field Research Director (FRD).

   b. If problems occur during application, refer to SOP 6.5.

   c. Any repairs or replacements will be documented as non-routine maintenance in the appropriate log(s).

7. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

SOP: 4.4, revision 10. Calibration and use of granular applicators.

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N. C. State University, IR-Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.5 **Revision Number:** 14

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Lisa Cutts **Date:** 1-25-2018

Title: Calibration, use, and cleaning of an air blast sprayer

Purpose: To determine the delivery rate of air blast sprayer and make adjustments as necessary to ensure an accurate application of the test substance

Scope: For use when calibrating an air blast sprayer for applying test substances to tree/bush crops.

Procedures:

1. Sprayer hoses, pipes, connections, pressure gages, pressure regulator and tank will be examined for leaks. The sprayer will be examined for worn belts and pulleys.

2. The sprayer will be calibrated to deliver a volume per area within the guidelines specified by the protocol. Always calibrate before initial test substance application and after any application parameter changes, if applicable. For multiple applications, recheck the calibration to confirm accurate delivery (e.g., $\pm$ 5% of initial calibration or as specified by the protocol)

3. To calculate speed of sprayer, in miles per hour (MPH), the following formula will be used:

$$MPH = \frac{(D/Sec)}{1.47}$$

Where D is distance of tract used measured in feet and Sec is seconds.

4. With air blast sprayers, nozzle spacing (NS) will be determined by dividing row spacing by the number of nozzles used and dividing that number by 2 if you will be applying test substance by making two passes per row (one on each side).

5. Gallons per minute (GPM) will be calculated by averaging the output, in milliliters (ml) from each nozzle for 15 seconds, multiplying this value by four and then dividing by 3785. As these amounts are immediately mixed and blown onto the crop after exiting the sprayer, the NCSU IR-4 Field Research Center does not set range limits regarding the consistency of output among the tips of an airblast sprayer.

6. Once application speed, in miles per hour (MPH), nozzle spacing (NS), in inches, and sprayer output, in GPM, are known, gallons per acre (GPA) can be calculated with the following formula:

$$GPA = \frac{GPM * 5940}{MPH * NS}$$

7. When the application is made to trees, the nozzle arrangement will be to direct approximately 2/3 of the spray pattern to the top one-half of the trees and approximately 1/3 of the pattern to the lower one-half of the trees. When the application is made to bush or cane crops that have fruit on all portions of the plants, the spray pattern should be equally distributed to entire plant.

8. Operator must carefully operate equipment, during application, under the same conditions as during calibration. Ensure solution is thoroughly mixed before application and continue to agitate during application.

9. The sprayer will be cleaned immediately after use by rinsing the tank with clean water. The sprayer will then be partially filled with ammonia plus water and operated to clean spray lines, tank, and nozzles. Finally, the tank will be partially filled with water and operated to clean ammonia residue from lines, tank, and nozzles. Cleaning of the sprayer will be documented in the maintenance records for the sprayer.

SOP: 4.5, revision 14. Calibration, use, and cleaning of an air blast sprayer.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.6 **Revision Number:** 16

Submitted by: Roger B. Batts RBB **Date:** 1-9-16

Approved by: Linel **Date:** 1-29-2018

Title: Calibration of backpack sprayer for foliar test substance applications to erect crops.

Purpose: To determine the delivery rate of a backpack sprayer for accurate pesticide application when multiple nozzles are used to direct insecticides and fungicides on the crop.

Scope: For use when calibrating a backpack sprayer for applying directed sprays of test substances to erect crops (i.e., blueberry, tomato, pepper)

Procedures:

1. Always calibrate before initial test substance application. For multiple applications, recheck the calibration to confirm accurate delivery (e.g., ± 5% of initial calibration or as specified by the protocol), or recalibrate.

2. Prior to use, visually inspect hoses, pipes, fittings, regulators, pressure gages, and tanks for obvious wear or potential leaks and repair or replace as necessary. Record inspection and any maintenance performed in the appropriate log(s).

3. Refer to the protocol for any specified application requirements.

4. With directed applications to erect crops, nozzle spacing (NS) will be determined by dividing row spacing by the number of nozzles used and dividing that number by 2 if you will be applying test substance by making two passes per row (one on each side).

5. Gallons per minute (GPM) will be calculated by averaging the output, in milliliters (ml) from each nozzle for 15 seconds, multiplying this value by four and then dividing by 3785. Output from each nozzle will not vary by more than 5% from highest to lowest. Any part(s) contributing to values outside this range will be cleaned or replaced. This procedure will be repeated until output of all nozzles fall within required parameters.

6. Once application speed, in miles per hour (MPH), nozzle spacing (NS), in

inches, and sprayer output, in GPM are known, gallons per acre (GPA) can be calculated with the following formula:

$$GPA = \frac{GPM * 5940}{MPH * NS}$$

7. Speed will be determined by timing (sec) the movement of the sprayer over a known distance (D) or by selecting a speed, calculating the needed pass time, and adjusting until pass time is matched. This can be done by using the following formula:

$$MPH = \frac{(D/sec)}{1.47}$$

8. Operator must carefully operate equipment, during application, under the same conditions as during calibration. Ensure solution is thoroughly mixed before application and continue to agitate during application if possible. The test substance must be applied uniformly to the entire crop.

9. Application container/tank will be triple-rinsed with water, then rinsed with ammonia, then triple-rinsed with water again. Spray system will be flushed through with an ammonia/water mixture, then flushed through with water alone. Cleaning should be recorded in the appropriate log(s).

10. Remedial action to be taken in case of failure or malfunction should include:

    a. Immediately report malfunction to the Field Research Director (FRD).

    b. If problems occur during application, refer to SOP 6.5.

    c. Any repairs and replacement of parts, other than changing nozzles and/or strainers while creating a different setup of the boom, will be documented as non-routine maintenance in the appropriate maintenance log(s).

    d. Changes of nozzles and/or strainers while creating a different setup of the boom will be documented as routine maintenance in the appropriate log(s)

11. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

SOP: 4.6, revision 16. Calibration of backpack sprayer for foliar pesticide applications to erect crops.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.7 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linli Cai **Date:** 1-29-2018

Title: Operation and maintenance of farm equipment.

Purpose: To assure that the crop or commodity under study is grown under simulated commercial conditions, in a quantity sufficient for the trial and in a good state of health.

Scope: All locations where the farming operations are performed for trials are conducted under good laboratory practices (GLPs)

Procedures:

1. The maintenance records for any equipment used to prepare soil, cultivate, apply fertilizer, and irrigate the crop where the trial is conducted will be kept by the research station personnel and will be the only records available on that equipment.

2. Just prior to the initiation of the use of the equipment (tractor, plow, disk, harrow, planters, harvester etc.) it will be visually inspected to see that it is in good working order, properly lubricated, and in good mechanical condition.

3. Any necessary repairs or adjustments should be made prior to the use of the equipment in the GLP trials.

4. The operator of the equipment should be familiar with its operation and safety precautions.

5. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

SOP: 4.7, revision 9. Operation and maintenance of farm equipment.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.8 **Revision Number:** 11

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei [signature] **Date:** 1-29-2018

Title: Calibration of field instruments.

Purpose: To assure that all instruments and devices used in good laboratory practices (GLP) trials are accurate and in good working order.

Scope: All facilities where GLP trials are conducted.

Procedures:

1. Each GLP-maintained instrument (e.g. min/max thermometers, thermographs, etc.) should be tested annually to determine that it is within the desired tolerance. Minimum/maximum thermometers and thermograph calibrations and standardization will be done according to the following procedures:

    a. Accuracy of minimum/maximum thermometers will be tested by placing the thermometer into an ice bath. The gauge should read near 32 F. Acceptable tolerance is ± 2F.

    b. Accuracy of minimum/maximum thermometer will also be tested by comparing ambient temperature with a thermometer that has been confirmed accurate according to NIST standards. Acceptable tolerance is ± 2 F.

    c. Thermograph will be calibrated by placing a thermometer that has been calibrated according to section 1.a. and 1.b. beside the thermograph and allowed to equilibrate. The thermograph and thermometer should be within 2F of each other.

2. A written record of the dates and results of the tests and of the acceptable tolerance for each instrument should be kept in the appropriate log.

3. Those instruments and/or devices that give inconsistent results or are not accurate to within desired tolerances should be replaced.

4. If a manual is not available to describe how an instrument or device should be tested, record the testing procedure used in the relevant log or describe in a standard operating procedure (SOP).

5. Personnel currently operating the equipment are responsible for the maintenance and remedial action taken in case of malfunction.

SOP: 4.8, revision 11. Calibration of field instruments.

Reviewed By:	Date:

N. C State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.9 **Revision Number:** 11

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linel Cox **Date:** 2018-01-29

Title: Procedure for operation, calibration, and maintenance of thermograph.

Purpose: This document is for use by IR-4 personnel to define techniques used for calibrating, operating, and maintaining thermographs at the NCSU IR-4 Field Research Center.

Scope: This SOP describes the proper procedures used by IR-4 personnel to ensure accurate calibration, operation, and maintenance of thermographs.

Procedures:

1. To activate unit turn on the power switch. Set for using daily, weekly, or monthly removable charts.
2. Label chart and indicate start with initials and date.
3. When the reading period is over, remove chart and replace with a new one. Record termination date and initials.
4. These charts will be deemed raw data and handled as such at all times.
5. Units will be appropriately maintained. If the unit fails, it will be serviced by a technician. The only exception is the changing of batteries.
6. All malfunctions, calibration, and maintenance will be documented in the appropriate log
7. All units will be calibrated/standardized on at least an annual basis, using the following procedure:
   a. Place a calibrated thermometer beside the unit to be calibrated.
   b. Allow at least 15 minutes to assure thermometer equilibrium.
   c. Observe and record the temperature values of the thermometer and

the recording thermograph into the appropriate log.

d. The difference in temperature observed by these thermometers from the thermograph must not be greater than 2 F.

e. If the temperature difference is greater than 2 F, adjust the thermograph.

f. If the above correction fails, a service call should be requested for the unit. Another appropriate monitoring device will be used as a replacement until the unit is repaired.

g. Original thermograph charts will be archived per procedures described in SOP 9.5.

8. Personnel conducting calibration or maintenance of thermograph are responsible for documentation of actions taken.

SOP: **4.9, revision 11.** Procedure for operation, calibration, and maintenance of thermograph.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.10 **Revision Number:** 6

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Curtis **Date:** 1-29-2018

Title: Calibration and use of Nikon Monarch® Laser 800 optical range-finder.

Purpose: To establish guidelines for the calibration and use of a Nikon Monarch® Laser 800 in order to accurately measure distance related to the establishment of IR-4 magnitude-of-residue trials at the NCSU IR-4 Field Research Center.

Scope: All facilities where trials are conducted under good laboratory practices (GLPs).

Procedures:

Calibration: Calibration of the Nikon Monarch® Laser 800 optical range-finder shall be done annually to determine if it is within the desired tolerance.

1. Ensure that the unit displays information when activated by pressing the POWER button. If so, continue to Step 3. If no display occurs or the battery image is blinking or not present, continue to Step 2.

2. Check and replace battery, if necessary

3. Using a tape measure or other accurate measuring device, establish two 'close-range' known distances. One should have a distance that measures in whole yards (i.e., 10.0 yds) while the other should have a distance that measures in 0.5 yards (i.e., 10.5 yds). A third 'long-range' distance will also be established (i.e., 100 yds). Place an object at each distance that is large enough to easily find in the optical range-finder crosshairs. Ensure that the object has a flat surface that will reflect the laser directly back to the optical range-finder. Avoid bright-colored objects and/or extremely bright conditions during this operation, as the reflectance in these circumstances may produce an incorrect readout from the device.

4. Make sure the display is reading in yards. If meters are displayed, hold the MODE button down for more than 1.5 seconds. This will switch the units.

5. Record distance values from the range-finder display.

a. Stand with range-finder at one end of the pre-measured distance for whole yards, aim at the object placed at other end of this distance and press the POWER button to gain a read out of the distance. Repeat at least once for consistency and accuracy. Record the displayed value in the appropriate log. If values for whole yards do not match the measured distance, do not use range-finder for determining distances. Use some other device.

b. Stand with range-finder at one end of the pre-measured distance for half yards, aim at the object placed at other end of this distance and press the POWER button to gain a read out of the distance. Repeat at least once for consistency and accuracy. Record the displayed value in the appropriate log. If values for half yards do not match the measured distance, do not use range-finder for determining distances. Use some other device.

Use:

1. To determine the distance to an object, do the following:

   a. Press the POWER button to turn on the unit.

   b. Make sure the object is large enough for the range-finder to detect and display a reading. If this is not the case, place an object that is large enough to be detected at/on the initial object. Be sure not to create angles of deflection when placing the second object (see Calibration, Step 3, above).

   c. While viewing the object through the range-finder, press the POWER button to create a displayed distance. Repeat at least once for consistency and accuracy. Record the distance in the appropriate place.

Safety Precautions:

1. Never look directly at the laser beam or directly at the sun when using the range-finder.
2. Do not operate with other optical elements, such as binoculars, lenses, etc.
3. Do not disassemble unit.
4. If unit's body cover is damaged, or if it emits a strange sound due to dropping or from some other cause, immediately remove the battery and stop using.
5. Do not press POWER button when not using the unit.
6. Do not leave within reach of small children.

7. Remove water, sand, or mud immediately with soft, clean, dry cloth.
8. Do not attempt to use range-finder under water.
9. Do not swing by lanyard or leave in unstable situation. Both could result in damage to the unit.
10. Do not leave unit in direct sunlight or in situations of extreme heat.

SOP: 4.10, revision 6. Calibration and use of Nikon Monarch® Laser 800 optical range-finder.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.11 **Revision Number:** 2

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Lisel Cuff **Date:** 1-29-2018

Title: Operation, maintenance, and cleaning of borrowed or seldom-used equipment.

Purpose: To assure that borrowed equipment is operated, maintained, and cleaned appropriately for the conduction of good laboratory practice (GLP) trials.

Scope: All locations where borrowed equipment is required to conduct GLP trials.

Procedures:

1. Upon receipt of equipment, ensure that any available owners/operation manuals are reviewed and understood. Receipt of equipment is to be documented in the appropriate log.

2. Visually inspect the equipment to ensure that it is in good working order. Any discovered problems should be repaired prior to use. These repairs are to be documented in the appropriate log

3. Prior to use, any surface of the equipment that may contact the test substance or target should be cleaned. This cleaning is to be documented in the appropriate log.

4. Perform required task(s) with the equipment.

5. After each use, the equipment is to be cleaned according to the equipment owner's manual or an appropriate standard operating procedure (SOP). This cleaning and any SOP that was used shall be documented in the appropriate log.

6. Document return of the equipment in the appropriate log.

SOP: **4.11, revision 2.** Operation, maintenance, and cleaning of borrowed or seldom-used equipment.

Reviewed By:	**Date:**

N. C State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 4.12 **Revision Number:** 4

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei Gao **Date:** 1-29-2018

Title: Operation, cleaning, and maintenance of forced-air drying ovens.

Purpose: This document is for use by IR-4 personnel to define procedures used for operating, cleaning, and maintaining the forced-air drying ovens at the NCSU IR-4 Field Research Center.

Scope: This standard operating procedure (SOP) describes the proper procedures used by IR-4 personnel to ensure proper operation, cleaning, and maintenance of the forced-air drying ovens at the NCSU IR-4 Field Research Center.

Procedures:

I Operation

1. Inspect each unit and review the maintenance log for each to ensure unit trays and other surfaces were cleaned, according to this SOP, after previous use. If there is any question on whether or not cleaning was performed, the unit will be cleaned prior to operation.

2. To activate unit turn on the power switch(es) for the oven(s) to be used. Use a separate drying oven for each treatment from which samples were collected. Set each oven to the desired drying temperature, per protocol, by using the appropriate button or dial and allow unit to reach this temperature. While temperature is adjusting, verify proper operation of each unit (good air flow from exhaust, normal operational sounds, etc.). If there is any concern about the operation of a unit, shut it off and contact service provider. Any repairs and maintenance to drying ovens will be documented in the appropriate NCSU IR-4 Field Research Center logs.

3. Personnel detecting a problem or malfunction of a drying oven are responsible for documenting both the problem and the repair in the appropriate NCSU IR-4 Field Research Center logs. If another unit needs to be used, follow steps 1 and 2 for the new unit.

4. Line each drying tray that will be used with a single layer of paper to prevent

portions of the samples from falling from the tray. Mark each paper with the corresponding sample identification number or letter, to reduce chances of confusion and/or contamination.

5. To dry samples, follow the procedures described in NCSU IR-4 Field Research Center SOP 8.4, "Forced-air drying of RAC (Raw Agricultural Commodity) Samples". All sample drying will be documented in the appropriate NCSU IR-4 Field Research Center logs.

II Cleaning

1. After drying is complete and samples removed, all paper liners are to be discarded. Retention of paper liners may lead to contamination of subsequent samples.

2. Drying trays are to be cleaned by:
   a. removing the trays from the drying oven(s)
   b. rinsing each tray thoroughly with ammonia
   c. rinsing ammonia residue off with a thorough water rinse
   d. air-drying trays completely prior to replacing them into drying oven(s)

3. While trays are air-drying, the interior surfaces of the oven(s) will be cleaned by:
   a. sweeping or vacuuming out any debris
   b. wiping surfaces with clean rag(s) carrying ammonia + water
   c. wiping surfaces with clean rag(s) carrying water only
   d. wiping surfaces with clean, dry rag(s)

4. Cleaning of drying oven(s) will be documented in the appropriate NCSU IR-4 Field Research Center logs.

III Maintenance

1. Each drying oven will be calibrated and serviced on an annual basis, using local dealer/distributor of the drying oven(s). All maintenance activities will be documented in the appropriate NCSU IR-4 Field Research Center logs. Any documents related to professional calibration of the drying oven(s) will be kept in the NCSU IR-4 Field Research Center archives.

SOP: 4.12, revision 4. Operation, cleaning, and maintenance of forced-air drying ovens at the NCSU IR-4 Field Research Center.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 5.1 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei [illegible] **Date:** 1-29-2018

Title: Commodity production and maintenance.

Purpose: Assure that commodities are grown under best management practices.

Scope: All locations developing data on test crops.

Procedures:

1. Refer to an up-to-date publication on the production of the commodity under trial. If no such publication exists, consult with agricultural specialist (i.e., University extension specialist, county/regional extension agent, grower, etc.) familiar with the production practices for the commodity.

2. It is imperative that any maintenance activities in trials conducted under good laboratory practices (GLPs) be performed in ways that will not impact sample integrity

3. A soil sample will be obtained and analyzed for soil texture, organic matter, cation exchange capacity and pH from the trial site.

4. Lime, fertilize and/or condition the soil at the site as necessary to bring the soil within the requirements of the commodity. This will normally be performed by research station personnel or grower, depending of the trial location.

5. Apply appropriate maintenance pesticides (preplant herbicide, soil insecticide, fungicide drench, soil-incorporated nematicide etc.) as required. Document maintenance chemicals in the field raw data notebook.

6. If maintenance pesticides are applied to the commodity, they should be applied according to the label directions. For residue trials, no pesticide should be applied that would interfere with the chemical analysis of the pesticide under evaluation. If in doubt, consult the analytical chemist, analytical laboratory or study director identified in the protocol to determine if a maintenance chemical may be used.

7. Perform other cultural practices as necessary to establish and maintain the commodity.

SOP: 5.1, revision 12. Commodity production and maintenance.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 5.2 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Carter **Date:** 1-29-2018

Title: Method for seeding or transplanting.

Purpose: Help ensure that an appropriate and high quality crop is produced in trials conducted under good laboratory practices (GLPs).

Scope: All locations developing data on test crops.

Procedures:

1. Determine the correct species and variety to use as specified by the study protocol. If the variety is not specified, select a variety commonly used in the area by commercial producers. With transplanted crops, plants as uniform in growth and color as possible will be used.

2. Determine within and between row spacing and seed/transplant depth as specified in cooperative extension services recommendations.

3. If exact seeding rate cannot be attained due to seed size and/or equipment capabilities, thinning of the emerged crop to the proper population will be performed.

SOP: 5.2, revision 9. Method for seeding or transplanting.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 5.3 **Revision Number:** 8

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cuff **Date:** 1-29-2018

Title: Determining yield or quality.

Purpose: To assure that a measurement of yield or quality of the various treatments is taken if required to evaluate the effects of the treatments.

Scope: All locations conducting trials where the protocol requires yield data. (Handling of residue samples is covered under NCSU IR-4 Field Research Center SOP 8.1).

Procedures:

1. Check the protocol for information on time of harvest. If none, follow commercial practices in the area for the time of harvest of the commodity. These practices should be documented in the raw data notebook.

2. Where grading standards are known or exist, the harvested commodity should be graded, accordingly.

3. Each portion of the commodity, divided as to its quality standard, should be weighed or measured to determine yield. Written records should be kept of each measurement for each plot.

4. Various methods are utilized by various researchers to harvest a commodity. The method used, if not specified in the protocol, should be recorded in the raw data notebook.

SOP: 5.3, revision 8. Determining yield and quality.

Reviewed By:	Date:

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Effective Date: Date of Approval

SOP#: 6.1 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cash **Date:** 1-29-2018

Title: Adding a test substance concentrate to a carrier in the spray tank of a sprayer.

Purpose: To obtain the proper dilution and mixing of the concentrate in the spray tank.

Scope: All locations conducting good laboratory practice (GLP) trials

Procedures:

1. After the sprayer has been inspected and calibrated, empty the water from the tank.

2. Measure the amount of water needed for the application into a separate container. Make sure this amount is sufficient to cover the entire plot. Make sure the chosen spray tank will hold this amount and the calculated amount of test substance concentrate needed.

3. For some formulations (i.e. wettable powder), it may be necessary to make a slurry mix first by adding the concentrate to a small volume of water in a separate, clean container prior to placing in the spray vessel. To do this, remove a generous amount of water from the calculated amount for total mix. Add the slurry to the water in the spray tank. Using the remainder of the water that was removed earlier, thoroughly rinse the container that held the slurry into the spray tank.

4. Add the remaining portion of the removed water to the spray tank, so that final volume and mix match the mix calculations. Close and tighten the lid. Rinse the outside surface of the spray tank with clean water, if needed.

5. Agitate the spray mix before and during application to insure an even mix of the test substance and water.

SOP: 6.1, revision 12. Adding a test substance concentrate to a carrier in the spray tank of a sprayer.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 6.2 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda A/B **Date:** 1-29-2018

Title: Procedures for the application of the trial test substance(s) in the field and greenhouse.

Purpose: To assure that the trial pesticide(s) are applied uniformly to the plots.

Scope: All locations conducting field trials

Procedures:

1. Ensure all settings of pressure, speed, granular flow etc. are set according to specification from the calibration as previously performed.

2. Just before entering each plot make sure you are travelling at the correct speed and turn on the sprayer or release the granules. Maintain the correct speed for each pass through the plot.

3. Apply the material according to the directions in the protocol or as specified on the label. If fumigants or mist blowers are used, follow instructions of the manufacturer of the equipment. If a fumigant is used, two people are required. One will do the actual application and one will observe from a safe place to provide rescue assistance if necessary.

4. Calculations should be made to minimize the amount of spray material left in the spray equipment. If the material being applied is US EPA registered, unused spray material should be applied to an overplanting of the crop at a distance adequate to prevent contamination of the test plot by drift or downslope movement of water. In the event that no overplanting of the crop exists, unused material may be placed along the field edge so long as contamination prevention measures listed above are followed.

SOP: 6.2, revision 10. Procedure for application of the trial test substance(s) in the field and greenhouse.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 6.3 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Culpepper **Date:** 1-29-2018

Title: Cleanup of application equipment.

Purpose: To assure that pesticide application equipment is decontaminated without adversely affecting personnel or the environment.

Scope: All locations where pesticides are used.

Procedures:

1. Granules-Remove any excess granules and return them to the original container if this procedure does not affect the integrity of the contents or dispose of the excess by using appropriate methods for handling hazardous wastes. Record in the appropriate log the amount of granular material used in the trial(s).

2. Liquids-If labeled, unused spray material should be applied to an overplanting of the crop at a distance adequate to prevent contamination of the test plot by drift or downslope movement of water. In the event that no overplanting of the crop exists, unused material may be placed along the field edge so long as contamination prevention measures listed above are followed. If excess spray material is applied to an overplanting of crop, this area will be marked as treated and consumption prohibited.

3. In a suitable area away from aquatic areas or danger of aquatic contamination, rinse the granular applicator to remove pesticide dust from the inside and outside. After application of a liquid mixture, the container/tank will be triple-rinsed with water, then rinsed with ammonia, then triple-rinsed with water again. The entire spray system will be flushed through with an ammonia/water mixture, then flushed through with water alone. Cleaning should be recorded in the appropriate log(s).

4. Follow the disposal procedures for pesticide rinse water in accordance with current policies and guidelines of the state.

5. Properly dispose of expendable protective clothing. Clean nondisposable

items such as respirators, face shields, and other equipment by following the manufacturer's instructions or with soap and water as appropriate.

6. After the application equipment is dry, lubricate those parts requiring lubrication and return the equipment to storage.

SOP: 6.3, revision 12. Cleanup of application equipment.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 6.4 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cuff **Date:** 1-29-2018

Title: Handling the test substance.

Purpose: To explain the procedures required in the receipt, removal, use, return and transfer of the test control and reference substances.

Scope: All locations where pesticides are used. For the purposes of this SOP, test substance also applies to control and reference substances.

Procedures:

1. Upon notification of test substance arrival at receiving point, NC State IR-4 personnel will collect test substance as soon as possible, preferably within 24 hours.

2. The test substance should be stored in the pesticide storage facility until it is needed for use in the trial(s). When a test substance is removed or transferred to a different location, removal date, return date, and the amount removed are to be recorded. The temperature range that the test substance is exposed to while out of storage will also be recorded. Removal of test substance prior to the day of use is allowable so long as proper documentation, according to item #4 below, is performed.

3. The storage temperatures of the test substance should be recorded in the raw data. A thermograph with a 30-day chart will be used for continuous monitoring of pesticide storage temperature. Calibration of the thermograph is outlined in SOP 4.9.

4. When a test substance is removed from storage, the following should be recorded in the test substance use log:

    a. complete trial number
    b. test substance name and lot/batch number
    c. removal date and initials
    d. temperature range while out of storage
    e. return date and initials

f. amount removed and initials

5. The original containers for all GLP test substances must be returned to the manufacturer or retained until completion of the study and/or permission is given by the study director to dispose of the containers. More guidance on IR-4 test substance container disposal can be found at:

http://ir4.rutgers.edu/Other/Advisories/advisory2005-01.pdf

Researchers may also visit

http://ir4.rutgers.edu/FoodUse/Food_UseSimple3.cfm

to obtain a listing of containers that he/she may discard.

SOP: 6.4, revision 13. Handling the test substance.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 6.5 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cato **Date:** 1-29-2018

Title: Procedures to follow if a problem occurs in the application of the test substance.

Purpose: To explain the procedures required in the event of a malfunction during the application of the test substance.

Scope: All locations where test substances are used. For the purposes of this SOP, the term 'test substance' also applies to control and reference substances.

Procedures:

1. During application, the operator should observe the process to make sure that the test substance is being evenly distributed to the commodity.
2. In the event of a malfunction (i.e., a nozzle is plugged or a hose breaks), the operator should take immediate action to correct the situation.
3. The affected portion of the plot should be flagged.
4. Appropriate individuals (e.g., the study director) should be notified of the incident, details recorded in the raw data notebook, and maintenance records retained in the appropriate log(s).

SOP: 6.5, revision 10. Procedures to follow if a problem occurs in the application of the test substance.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 7.1 **Revision Number:** 8

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Cutts **Date:** 1-29-2018

Title: Collection of raw data electronically.

Purpose: To assure that raw data collected electronically are verifiable if audited.

Scope: All locations conducting field or greenhouse trials.

Procedures:

1. Check the power supply on portable units to see that it will be adequate during the data collection and data transfer period.

2. Make sure the correct program for data collection is ready and available for use.

3. At the beginning of data collection, provide verification that the system is working by collecting data from the first plot electronically and also have someone record the data by hand. At the end of the data collection period, the printout of the electronic data and the hand-collected data should be signed by the person collecting the data. If both sets of data are in agreement a signed and dated statement to that effect should be written in the maintenance log.

4. Prompts should be used as much as possible to avoid any confusion in collecting the data. Where feasible, the prompts should state the plot # from which the data is being collected, the current date, and the type of data being collected.

5. Data should be taken in an orderly fashion so as to avoid any confusion.

6. At the end of the data collection period, the data should be transferred to a storage system and immediately printed out with appropriate identification. This hard copy must be dated and signed then stored in the trial(s) file folder.

7. All remote sensing and other automatic data collecting and/or recording

devices should be inspected and calibrated.

8. Prints of data from these devices must be legible to persons with normal vision and dated and signed when printed or plotted.

9. Hard copies of computerized data and/or other written or plotted date sheets must be dated and signed, and retained in the file folder of the project.

10. Each data sheet from a monitoring device should be marked in ink with the name of the trial ID number, dates (day, month, year) of occurrence of the event measured, units of measurement and signed and dated by the person preparing the data sheet.

SOP: 7.1, revision 8. Collection of raw data electronically.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 7.2 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linli Cui **Date:** 1-29-2018

Title: Recording of raw data.

Purpose: To assure that raw data collected and recorded are accurate and available for audit.

Scope: All locations conducting trials.

Procedures:

1. All raw data will be recorded in indelible ink.

2. Changes to the raw data should be made by drawing a single line through the original entry so as not to obscure it. The date, signature (or initials) and reasons for change (brief description or error code) must accompany any change. Acceptable error codes include:

ME = Measurement Error	CE = Calculation Error
SP = Spelling Error	EE = Entry Error
WE = Wrong Entry	NR = Not Recorded
IE = Illegible Entry	IW = Inappropriate Word
TE = Transcription Error	AW = Accidental Writeover
UE = Unnecessary Entry	PE = Pagination Error
LE = Late Entry	NA = Not Applicable
NI = New Information	

Other error codes can be used, however, the codes must be noted in the IR-4 Field Data Book.

3. Pages containing raw data shall not be discarded.

4. Cross-reference instrument or statistical printouts when such data are retained in a separate location.

5. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data.

6. Make sure that all data required by the study protocol or by the forms provided in the field data book are collected and recorded.

SOP: 7.2, revision 9. Recording of raw data.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 7.3 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Lisa Cuff **Date:** 1-29-2018

Title: Calculations for data presentation.

Purpose: To establish guidelines for computation and presentation of data.

Scope: Field sites conducting trials under the minor use pesticide program.

Procedures:

1. Results must be reported to correct number of significant figures reflecting an appropriate level of certainty.

2. In carrying measured quantities through calculations, the following rules are used:

   a. Multiplication and division: the result must be rounded off as having no more significant figures than the measurement with the fewest significant figures.

   b. Addition and subtraction: the result is rounded off to the same number of decimal places as that of the term with the least number of decimal places.

3. Round-off rules:

   a. If the first digit to be dropped is less than 5, round down.

   b. If the first digit to be dropped is greater than or equal to 5, round up.

4. When a manual calculation involves two or more steps, retain at least one additional digit (insignificant figure) for intermediate answers. Round off at the end.

5. When using computer(s) or calculator(s), serial calculations should be done with unrounded numbers and the final result is to be rounded.

SOP: 7.3, revision 10. Calculations for data presentation.

Reviewed By:	Date:

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Effective Date: Date of Approval

SOP#: 7.4 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cuff **Date:** 1-29-2018

Title: Method for collecting efficacy and phytotoxicity data.

Purpose: To describe the procedure used for taking biological field data.

Scope: All locations conducting field trial(s).

Procedures:

A. Phytotoxicity data:
Consult the protocol to determine the method and timing of the phytotoxicity data. If no method is cited then reference your method or proceed as follows:

1. Where possible, take phytotoxicity data within 48 hours after the treatment, 1 week later and at the termination of the trial(s). If symptoms occur during this period that warrant a reading, then additional phytotoxicity data should be taken as necessary.

2. Assign a phytotoxicity rating of 0 to 100 for each plot; 0 = plant healthy. 100 = plant dead. 1 thru 99 = the percentage necrosis, yellowing and/or stunting of the plants in each plot.

B. Pest data:
Consult the protocol to determine the method and timing of the pest data. If no method is cited then reference your method(s) for each pest or proceed as follows:

1. Where possible, take pest data within 48 hours after the treatment and at various intervals thereafter depending on the pest life cycle and at the termination of the trial(s).

2. Disease data - Record the name of the disease(s) being observed. Record the symptom(s) for each disease. Randomly select 5 plants in the middle row of each plot and record the severity of each disease in a rating system of 0 to 10 for each plant. Zero = plant healthy. Ten = plant dead. One

through nine = the percentage disease appearing on the plant. it there are less than five plants/row, record data from 5plants/plot or all the plants in a plot.

3. Insect data - Record the name of the insect (s) being observed, Record the damage symptom(s) for each insect.

   For damage symptoms - randomly select 5 plants plants in the middle row of each plot and record the severity of damage for each insect in a rating system of 0 to 10 for each plant. Zero = plant healthy. Ten = plant dead. One through nine = the percentage damage appearing on the plant. If there are less than five plants/row, record data from 5 plants/plot or all the plants in a plot.

   For insect pest population counts - take a random sample of the pest population (i.e. 5 leaves/plant of 5 plants/plot, 4 3-in diam. soil cores/plot, 100 apples/tree etc.) to insure an accurate reflection of the pest density/unit area.

4. Nematode data - Record the name of the nematode(s) being observed. Record the damage symptom(s) for each nematode.

   For damage symptoms - randomly select 10 plants in the middle row of each plot and record the severity of damage for each nematode on each plant using one of the rating systems described by the following:

   > Barker, K.R., J.L. Townshend, G.W. Bird, I.J. Thomason and D.W. Dickson. 1986, Determining nematode population responses to control agents. in Kickey, K.D. (ed.). Methods for evaluating pesticides for control of Plant Pathogens. Pages 283-296. (See article in Appendix)

   If there are less than 10 plants/row, record data from all the plants in a row.

   For nematode population counts-take a random sample of the pest population (i.e. root system of 2 plants/plot, 4 3-in diam. soil cores/plot, etc.) to insure an accurate reflection of the pest density/unit area as described by Barker et al., cited above. Use a method suitable to extract the nematodes from the soil or plant sample and cite the method here. Count and record the number of nematodes by the various life stages/unit of soil or root.

5. Weed data - Visually observe each plot and record the percentage (%) of the area (to the nearest 5%) covered by weeds. Record the names of the 5 most prominent weed species and percentage control of each species

relative to the non-treated check (to the nearest 5%) in each plot. Randomly place a grid covering an area of 1 ft^2 and divided by quadrants in the plot. Where possible, count the number of weeds in the grid. If weeds are too numerous, then count the number of weeds in the lower left quadrant, multiply by 4 and record this value as the number of weeds in the grid.

SOP: 7.4, revision 9. Method for collecting efficacy and phytotoxicity data.

Reviewed By:	Date:

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Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 7.5 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Catlin **Date:** 1-29-2018

Title: Experimental design and data analysis.

Purpose: To assure that all efficacy, yield, and phytotoxicity data developed are statistically sound.

Scope: All locations conducting trial(s)

Procedures:

1. The experimental design as specified by the protocol should be used. If none is designated, then the researcher should use a commonly accepted experimental design such as a complete randomized block design. The experimental design used should be documented in the raw data notebook.

2. A minimum of 3 replicates should be used (4 is preferred). No replicates or statistical analysis are required where the trials is for magnitude of the residue only.

3. Randomly assign the treatments.

4. Select an appropriate statistical package for data analysis and record sufficient information to identify the statistical package (i.e. Date, Revision no., Title, Authors, Source etc.) and determine the level of significance for the trial(s).

5. When the raw data are available for analysis, utilize the statistical package and follow instructions contained therein to conduct an analysis of variance and mean separation of the data.

6. Report the data as required by protocol, in tabular and/or narrative format.

7. Retain all data, analyses, notes etc. in the trial(s) folder with sufficient information to recalculate the data summaries and statistical analyses by another person without verbal input.

SOP: 7.5, revision 9. Experimental design and data analysis.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 7.6 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Cobb **Date:** 1-29-2018

Title: Data storage during the active life of the project.

Purpose: To assure that all data resulting from the trials is retained and usable.

Scope: All locations conducting trials.

Procedures:

1. It is the responsibility of the Field Research Director (FRD) to see that all raw data, summaries and other items connected with the trial are properly retained prior to sending the data to the Study Director (SD).

2. The FRD will see that all raw data, summaries, data logs, etc. connected with a trial are maintained during the active life of each project for which he/she is responsible. Afterwards, all raw data that is not directly used in a report is to be archived per procedures described in SOP 9.5. This also pertains to dated and signed hard copies of electronic data, computerized summaries etc. These should be placed in the file as soon as possible after the information is generated.

3. All notebooks, data sheets, summaries etc. should be clearly marked with the project identification number and any other information that may be needed to understand the data and its source.

4. Computer software or on line programs (i.e., SAS) used in the trial should be noted in the field data book (FDB) and information on the title, source, revision or other identifying information should be recorded and the data maintained and updated as needed and filed in the trial folder.

SOP:	7.6, revision 13. Data storage during the active life of the project.

Reviewed By:	**Date:**

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 8.1 **Revision Number:** 11

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Curtis **Date:** 1-29-2018

Title: Sample collection, identification, and records.

Purpose: To assure proper collection and identification of residue samples,

Scope: At locations where trials are conducted to obtain residue samples.

Procedures:

1. Consult the study protocol to establish specific dates and method for the collection of samples. If these dates are based on uncontrolled events (fruit size, spray applications etc.) then tentative dates should be established and refined as necessary. The Study Director (SD) and Quality Assurance Unit (QAU) should be kept informed when the dates are changed.

2. Collection of samples during periods of inclement weather should be avoided if possible.

3. Representative samples of the crop in each plot must be taken by a recognized procedure. Follow the protocol or record in the Field Data Book (FDB) the procedure used to ensure a representative sample.

4. Consult the study protocol to determine sample size and special instructions for the commodity.

5. Sample each treatment individually beginning with the untreated plot(s) and progressing through to the highest dosage.

    a. If sampling in this manner is not possible, cross-contamination of samples must be avoided and methods used to prevent cross-contamination should be thoroughly described in the FDB.

    b. Samples may also be simultaneously collected from different plots by different persons. If this is done, it should be clearly explained in the FDB.

c. Samples from each treatment should be individually packaged and labeled.

6. Take special care to do the following in the sample collection process:

   a. Avoid contamination of the field samples with the test substance during the sampling, labeling, storage and shipping processes. Take care not to allow the outer surface of the sample bag to contact treated crop during harvest

   b. Avoid taking diseased or undersized crop parts.

   c. Take care not to remove surface residues during handling, packing or preparation.

   d. Be certain tools are clean.

   e. Do not remove any soil or plant parts or trim the commodity unless it is so specified in the study protocol (leave stem in cherry, outer leaves on lettuce, etc., unless specified otherwise in the protocol.)

7. Plastic-lined cloth sampling bags with an identification tag sewn into the bottom stitching are usually provided to GLP cooperators for sample collection. If these bags have not been provided, a sampling bag suitable to protect the integrity of the sample should be used.

   It is highly recommended that for 'juicy' commodities (berries, fruits and vegetables that have been sectioned to reduce sample weight, etc.), a large resealable bag be added inside the sample bag to prevent these juices from freezing into the cloth of the sample bag.

8. Prior to sample collection, obtain a sufficient number of sample bags to collect all the samples required by protocol.

9. Before entering the field, use waterproof ink to fill in the label attached to the bottom of the bag and indicate the study ID number and bag number on the tag if more than one is used for the plot sample. Use of pre-printed adhesive labels is permitted provided that all required information is present. If no tag has been provided, then label each sample bag with waterproof ink with the following:

   a. PR Number
   b. Commodity (Crop)
   c. Chemical
   d. Replicate Number

e. Date sampled
f. Application rate (# ai/A)
g. Investigator Name/Address/Phone Number
h. Container Number (if more than 1 container for a sample)

10. On a 3 x 5 card or similar material (the use of pre-printed labels for this identification is allowed provided that all required information is present), type or print the following for each sample bag:

a. PR Number
b. Commodity (Crop)
c. Chemical
d. Replicate Number
e. Date sampled
f. Application rate (# ai/A)
g. Investigator Name/Address/Phone Number
h. Container Number (if more than 1 container for a sample)

11. Place each card generated for Step 10 in a moisture proof container (i.e., resealable plastic bag) and place it inside the correct sample bag. This is an important step since the label on the outside of the bag may get lost during handling and transit.

If a resealable plastic bag is used, as indicated in Step 7, this card SHOULD NOT be placed inside the resealable plastic bag, as it may become frozen into the sample and create difficulties at the analytical laboratory.

12. Sample bags should be burst proof. Cloth laminated plastic bags are preferred.

SOP: 8.1, revision 11. Sample collection, identification, and records.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 8.2 **Revision Number:** 14

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei [signature] **Date:** 1-29-2018

Title: Residue sample packing and storage procedures.

Purpose: To assure the integrity of residue samples after collection.

Scope: All locations where residue samples are collected.

Procedures:

1. If samples require refrigeration or freezing prior to shipping to the residue laboratory, containers with ice or blue ice in sufficient quantity to preserve the samples prior to storage should be taken to the site. Otherwise cartons of sufficient size and burst proof strength to hold the samples should be used.

2. Carefully place the sample as it is collected (or cleaned according to protocol, if required) in the sample bag marked for that sample. Make sure that the labeling for inside the bag (as required by SOP 8.1) is enclosed with the sample.

3. Close the sample bag so as to prevent loss of the sample under reasonable storage, handling, and transportation conditions. Excess air should be expelled from the bag.

4. Place the sample bag in the appropriate container as determined in # 1 above. Physically separate treated and nontreated samples.

5. When sample collection is completed, the samples should be placed in storage as soon as reasonably possible.

6. Consult the study protocol for the method, temperature, and maximum length of time for storage, if listed. If specifications are not given in the protocol, samples will be stored in a freezer at <32 F and shipped as soon as possible.

7. Samples identified for post-harvest processing should be processed or

shipped to the processor as soon after collection as possible.

8. The storage temperature of the samples will be continuously monitored using a maximum/minimum thermometer, or other calibrated device, and will be recorded in a freezer temperature log.

9. The sample storage freezer(s) should have limited access. Lock and key are required for any room and/or building housing sample storage freezer(s).

10. A log should be maintained for the items inside the storage equipment (i.e. freezer, refrigerator etc.) indicating the trial number, sample ID number, collection date with initials and removal date with initials. Removal of the samples prior to shipment should be recorded in the log with the name or initials of the person removing them, what sample bags or parts thereof were removed, date removed and date returned.

11. Freezer temperature does not have to be recorded if no residue samples are being stored.

12. A ' Sensaphone 400 ' alarm system will be used to monitor the temperature range of the samples.

    a. At the NCSU IR-4 Field Research Center, this alarm will be solely for notifying personnel if the temperature reaches a level that risks the thawing of samples; not for generation of raw data. Raw data documentation at this location is addressed in #8 above.

    b. The alarm system will be tested at least every 2-3 months by removing the sensor from the freezer and allowing it to exceed 32F and trigger the notification process that has been programmed into it. These tests, including documentation of 'pass' or 'fail', will be recorded in the appropriate facility logs. These tests may be conducted while samples are in the freezer, since the Sensaphone 400 will not be generating raw data.

    c. In the event of a test 'failure', the operator is to refer to the Sensaphone 400 User's Manual located in the NCSU IR-4 Field Research Center archives for troubleshooting guidance. If no resolution of the problem can be achieved, call Sensaphone Technical Service Department (610.558.2700) for assistance.

13. If freezer malfunctions and samples cannot be maintained at the desired temperature, samples will be moved to a functioning freezer. Transfer of the samples to the functioning freezer should be well documented by recording the date of transfer, sample trial number, and number of samples

and the new location. Storage temperature in the new storage equipment should be monitored, including logbook entries, as stated above.

14. A log shall be kept for any and all freezer repairs or maintenance activities. These activities shall be denoted as Routine or Non-routine in the log.

15. Maintenance and repair activities shall include but not be limited to any electrical or structural activities as well as simple defrosting of the freezer(s). Defrosting will also be recorded in the log. Defrosting should be performed in the absence of samples. However, if samples are present when defrosting is necessary, all samples and blue ice will be removed. Samples will be transferred to another freezer and documentation of the transfer will be shown as described in # 13 above. Blue ice will be allowed to completely thaw and dry prior to replacement in the freezer so that no condensation is present that may create extra ice build up in the freezer(s).

16. Personnel conducting maintenance or repairs are responsible for documentation of actions taken.

SOP: 8.2, revision 14. Residue sample packing and storage procedures.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 8.3 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linli Gu **Date:** 1-29-2018

Title: Sample shipping procedures.

Purpose: To assure that residue samples are removed from storage and shipped to the residue laboratory with a minimal or no loss of integrity.

Scope: All locations where residue samples are stored.

Procedures:

1. For overnight shipments, make arrangements with the carrier for shipment of the samples and determine any special packing instructions that are required to preserve the sample integrity. Note any limits on quantity of dry ice that may be set by the carrier. Obtain insulated containers, if necessary, of sufficient size and quantity to hold the residue samples and dry ice (where required) in a sample:dry ice weight ratio of at least 1:3 and pack the samples and dry ice in the containers just prior to shipment. The containers should have sufficient bursting strength so as to withstand normal handling in shipping and storage. Air freight shipments should be made on Monday or Tuesday to avoid potential weekend layovers, and shipment during holidays should be avoided.

2. For freezer truck shipment, contact shipping company and arrange shipping date

3. Prior to shipping samples, notify the laboratory with trial number, shipment date and method of shipment including the carrier and carrier schedule.

4. Complete appropriate residue sample shipping forms and send them, via fax or e-mail, to the Study Director (SD), Regional Field Coordinator (RFC) and residue chemist.

5. Place copies of the completed residue sample shipping forms and any chain-of-custody forms that may be required in waterproof containers and place in each of the sample shipping containers.

6. Label each container with the following information:
   a. Study Identification Number
   b. Return Name and Address of the sender
   c. Name and Address of the residue laboratory receiving the samples
   d. Affix "Experimental Samples-Perishable" sticker on each carton
   e. Where used, affix "Dry Ice" on two sides of the container
   f. When appropriate, label as box ____ of ____.

7. Tie or tape lids of each container firmly in place.

8. Provide the carrier with the samples for shipment.

SOP: 8.3, revision 13. Sample shipping procedures.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 8.4 **Revision Number:** 3

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linel Gulf **Date:** 1-29-2018

Title: Forced-air drying of RAC (Raw Agricultural Commodity) samples.

Purpose: To assure that all forced-air dried samples meet protocol specifications.

Scope: This SOP describes the procedure that will be used at the NCSU IR-4 Field Research Center for drying commodities to meet protocol sample moisture requirements.

Procedures:

I DETERMINE DRY MATTER PERCENTAGE OF CROP

1. Turn on drying oven(s) and set to desired temperature, per protocol. Document this temperature and temperature calibration information for each oven used for inclusion in the Field Data Book (FDB). Drying information for PRE samples may be documented using NCSU IR-4 Field Research Center Form 8.4-A.

2. A few days ahead of Raw Agricultural Commodity (RAC) sampling, collect PRE samples. Collect at least two samples for each drying oven to be used. Identify PRE samples with a different nomenclature than the RAC samples of the trial. If protocol lists RAC samples alphabetically (A,B,C...), PRE samples should be identified numerically (1,2,3...). Sample identification must be maintained while samples are drying. Document the procedure for maintaining identification in the FDB. At the NCSU IR-4 Field Research Center, this is typically done by writing the sample identification onto the paper (See SOP 4.12) that lines the drying tray.

3. Record 'wet weight' of each sample.

4. Place each PRE sample in drying oven in a way to ensure maximum air flow through the PRE samples. At the NCSU IR-4 Field Research Center, this will typically be a screen-bottom tray with a paper lining. Document this configuration for inclusion in the FDB.

5. Monitor these samples and record the weights as they dry. When no appreciable change in sample weight occurs (1% or less), record final, 'dry weight' of PRE samples.

6. Dry matter percentage can now be calculated:

   a. The formula:

   ((wet weight - dry weight)/wet weight) * 100

   results in moisture percentage of the PRE sample

EXAMPLE
((400g wet sample - 100g dry sample)/ 400g wet weight) = 75% moisture

   b. The formula:

   100% - % moisture

   results in dry matter percentage of the PRE sample

EXAMPLE
100% - 75% moisture = 25% dry matter

II DETERMINE TARGET DRY WEIGHT FOR RAC SAMPLES

1. Using the dry matter percentage calculated from above, the following formula:

$$\frac{\text{(dry matter portion of PRE samples * wet weight of RAC sample)}}{\text{dry matter portions required by protocol}}$$

results in the acceptable dry weight range of the RAC sample.

EXAMPLE:
PRE sample dry matter percentage = 25
RAC sample is 4 lb and protocol requires dry matter of 80% to 90%

(0.25 * 4 lb)/.8 = 1.25 lb, the RAC sample weight at 80% dry matter.
and
(0.25 * 4 lb)/.9 = 1.11 lb, the RAC sample weight at 90% dry weight.

III DRYING THE RAC SAMPLES

1. Use temperature settings that match the setting used in Part I. Drying information for RAC samples may be documented using NCSU IR-4 Field Research Center Form 8.4-B.

2. Use separate ovens to dry samples from different treatments. Document oven information (brand, model, and serial number) for inclusion in the FDB.

3. Record 'wet weight' of each RAC sample and calculate desired 'dry weight' range for each, per protocol, using formula in Part II.

4. Sample identification must be maintained while samples are drying. Document the procedure for maintaining identification in the FDB. At the NCSU IR-4 Field Research Center, this is typically done by writing the sample ID onto the paper that lines the drying tray.

5. Place each RAC sample in drying oven in a way to ensure maximum air flow through the RAC samples. At the NCSU IR-4 Field Research Center, this will typically be a screen-bottom tray with a paper lining. Document this configuration for inclusion in the FDB.

6. Monitor each sample as it dries, until the weight reaches the desired range calculated for each RAC sample.

7. Verify moisture percentage of the sample:

   a. The formula

$$100 * \left[\frac{\text{(final RAC dry weight)-(RAC wet weight * Dry matter portion of PRE)}}{\text{(final RAC dry weight)}} \right]$$

   results in moisture percentage of the dry RAC sample

EXAMPLE:
RAC wet weight = 4 lb
Final RAC dry weight = 1.18 lb
Dry matter portion of PRE sample = .25

$$100 * \left[\frac{(1.18)-(4.0 * 0.25)}{(1.18)} \right] = 15.25\ \%\ \text{moisture}$$

b. The formula:

100% - % moisture

<u>results in dry matter percentage</u> of the RAC sample

EXAMPLE
100% - 15.25 % moisture = 84.75 % dry matter

8. All calculations and any forms used during the drying shall be inserted into the FDB.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 9.1 **Revision Number:** 13

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei Gu **Date:** 1-29-2018

Title: Raw data report forms.

Purpose: To review the forms used to report raw data.

Scope: All locations conducting IR-4 good laboratory practice (GLP) trials

Procedures:

1. IR-4 Headquarters or other trial sponsor will provide a Field Data Book (FDB) to each of the cooperators for each trial undertaken. Detailed instructions are provided in the book and in the IR-4 Field Data Book Guidance Document located on the IR-4 webpage: ir4.rutgers.edu/trainingadvisories.html

2. All forms should be filled out legibly and mistakes should be crossed with a single line, initialed, dated, and the reason for change given or an assigned code for reason. Suggested codes for these entries can be found in NCSU IR-4 Field Research Center SOP # 7.2.

3. Blank forms may be photocopied as needed.

4. The forms provided in the FDB should be filled out as completely as possible at the time the data is collected.

5. Each location should use the forms provided or develop new forms where needed. The new forms should be placed in the FDB.

6. If a particular form or section of the form does not require a response, make a diagonal line across this form or section. Initial and date the diagonal line. Empty fields of two or more lines require a diagonal line with initials and date.

7. For IR-4 sponsored trials, number each page (i.e. Part *X*, Page *Y*) within each section of the raw data book, where *X* is the section of the field data notebook and *Y* is the current page number in that section. For trials with

non-IR-4 sponsors, pagination will be done per sponsor requirements, if different from the IR-4 format.

SOP: 9.1, revision 13. Raw data report forms.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
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Effective Date: Date of Approval

SOP#: 9.2 **Revision Number:** 10

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Gu **Date:** 1-29-2018

Title: Handling completed report forms that transcend two or more trials.

Purpose: To explain how report forms can be completed for one trial and serve as raw data for other trials.

Scope: All locations conducting trials.

Background: Where a field research director (FRD) is conducting multiple trials during the year, there may be an opportunity to utilize one form for data that pertains to more than one trial. Examples may be logs of various types, weather data, and sprayer calibration/pesticide application where the same test substance is used at the same time at the same rates on two or more crops (i.e. preplant herbicide, foliar insecticide). There are provisions within the GLPs for substitution of verified copies for original records. However, the retention of all original raw data is also a requirement. The following procedure is designed to meet the GLP and FIFRA requirements where copies of data are used.

Procedure: Each copy that is to be used for data common to more than one trial should contain a notation as to the trial that has been designated as the one containing the original raw data. This should read: "NCSU IR-4 Center, TRUE COPY, Location of Original _____". The copy should be initialed, dated and placed in the Field Data Books (FDBs).

SOP: 9.2, revision 10. Handling completed report forms that transcend two or more trials.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 9.3 **Revision Number:** 14

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei GU **Date:** 1-29-2018

Title: Disposition of raw data from trials

Purpose: To assure that raw data are sent to the archives.

Scope: All locations conducting trials where the original raw data is not archived at IR-4 Headquarters.

Procedures:

1. The field research director (FRD) will make an exact copy of the original completed field data book (FDB); including correspondences, protocol deviation forms, logs, and any accompanying documentation such as weather charts etc. The original FDB will be forwarded by the FRD to the regional field coordinator (RFC).

2. The FRD will retain the true copy of the FDB from #1 at the field facility. This copy shall be authenticated by stamping all pages as TRUE COPY or preferably, by including an index that lists trial number, name of trial and number of pages in each section along with researcher's signature. This copy may be discarded only after a report on the data has been submitted to EPA or the related study is cancelled. Researchers may also visit IR-4 Advisory #2005-02 at http://ir4.rutgers.edu/Other/Advisories/advisory2005-02.pdf to obtain a listing of studies that have reached this stage.

3. The RFC is responsible for the review of the documents received in #1 for completeness and accuracy of reporting. The RFC will follow up to obtain any missing data or to correct deficiencies.

3a. Corrections made by the FRD will be done in the following manner:
   1. The page of concern will be copied form the facility copy of the FDB (see #1 above). The uncorrected page will be returned to the FDB copy.
   2. The required corrections are to be made to the copied page. Since this copy will likely be in black and white, it is suggested that corrections be made in blue ink in order to distinguish the

corrected items. Corrections are to be made following the procedure set forth in SOP #7.2.

3. The corrected page will be repaginated in order to distinguish it from the unaltered page during subsequent data review. This shall be done by adding an alphabetic code following the page number (i.e., Part 6 Page 1 will become Part 6 Page 1a). If, for some reason, the page number already has an alphabetic code, the next letter should be used when corrections are made (i.e., Part 6 Part 1a will become Part 6 Page 1b). It is suggested that this be done using blue ink in order to distinguish the corrected page.
4. The corrected page will be copied, stamped as a True Copy, and placed behind the uncorrected page in the facility copy of the FDB.
5. The corrected page will be sent to the RFC for insertion at the corresponding location in the original FDB. The addition of the corrected page will be documented on the NCSU IR-4 page number index for the facility copy of the FDB (see #3b, below).
6. Steps 4 and 5, above, will ensure that the facility copy of the FDB is identical to the original FDB and will reduce confusion if any future corrections are required.

3b. Corrections that, with permission of the FRD, are made directly on the original page by the RFC do not require repagination. However, a copy of the page is to be sent to the FRD in order to ensure that the facility copy of the FDB is identical to the original FDB and will reduce confusion is any future corrections are required. The FRD will replace the corresponding uncorrected page with the corrected page in the facility copy of the FDB.

3c. The RFC will be forward the original FDB to the quality assurance unit (QAU). After QAU review and correction following steps 3a and 3b, if any, the original FDB will be sent to the study director (SD).

4. Any original document that is copied, in part or in total, to help complete raw data requirements of a field trial will be archived at the NCSU IR-4 Field Research Center per procedures described in SOP 9.5.

SOP: 9.3, revision 14. Disposition of raw data from trials.

Reviewed By:	Date:

N. C. State University IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 9.4 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linel GWB **Date:** 1-29-2018

Title: Retention of data.

Purpose: To assure that all data and documents connected with good laboratory practice (GLP) trials are archived.

Scope: All locations conducting GLP trials

Procedures:

1. The field research director (FRD) will see that the trial file containing the raw data, originals or true copies of reports, logs, etc. are submitted to the study director (SD) and that a true copy or original of any site-specific documents are retained at the field facility to assure that raw data is not lost. These retained documents are to be archived per procedures described in SOP 9.5. Per federal requirements (CFR40, Title 40, Chapter I, Subchapter E, Part 160.195), all archived data and documents are to be retained for five years or the life of the registration, whichever is longer.

2. The FRD should maintain a file and items placed in the file should be identified as to the trial they pertain to or the dates when the items were in use. The following is a list of information that should be retained:

   a. True copies of raw data including pest counts, yield, phytotoxicity, weather records, logs of instrument calibration and test substance receipt, distribution, etc.

   b. Copies of summaries including calculations and copies of information used from referenced sources.

   c. Copies of reports and correspondence related to the conduct of the trial.

   d. Copies of completed forms used during the trial and for summaries of the trial data.

e. Historical standard operating procedures (SOPs).

f. Master schedule of all GLP trials conducted at the facility.

g. Organizational charts, training records, job descriptions and CVs (current, out of date, or former employees).

h. Copies of computer software and/or information sufficient to identify outdated computer software or programs that were used in trial so data developed from these programs can be repeated if necessary in the reconstruction of the trial.

i. Any samples as required by the study protocol or the SD.

SOP: 9.4, revision 12. Retention of data.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 9.5 **Revision Number:** 3

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Gu **Date:** 1-29-2018

Title: NCSU IR-4 Field Research Center Archives

Purpose: To assure that any original data and/or records used in IR-4 trials that are kept at NCSU are protected and trackable.

Scope: NCSU IR-4 Field Research Center original data and records

Procedures:

1. The NCSU IR-4 Field Research Center archives are located in fire-prof cabinets housed in Room 110, 520, Brickhaven Dr. Raleigh, NC 27606

2. The NCSU IR-4 Field Research Center field research director (FRD) is responsible for the archives and will designate who shall have access to the archives. These individuals, along with initial permission date and permission termination date, will be noted in the archive log.

3. A log will be used to record removal and return of records to and from the archives.

4. Per federal requirements (CFR40, Title 40, Chapter I, Subchapter E, Part 160.195), all archived data and documents are to be retained for five years or the life of the registration, whichever is longer.

SOP: 9.5, revision 3. NCSU IR-4 Field Research Center Archives

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
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Effective Date: Date of Approval

SOP#: 10.1 **Revision Number:** 14

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Gu **Date:** 1-29-2018

Title: Disposal of test substances.

Purpose: To assure that test substance concentrate, spray solutions, rinse water, and containers are disposed of with minimal environmental contamination and in accordance with federal, state and local regulations.

Scope: All locations conducting field trials

Procedures:

1. Where institutional policies and guidelines do not exist, the following procedures should be followed.

2. Disposal of test substance concentrate and/or containers.

   a. Follow procedures in the protocol. Generally, containers cannot be disposed of under GLP until the study is completed. If it is necessary to dispose of the container prior to the end of the study, the study director must be consulted. More guidance on IR-4 test substance container disposal can be found at:

   http://ir4.rutgers.edu/Other/Advisories/advisory2005-01.pdf

   Researchers may also visit

   http://ir4.rutgers.edu/FoodUse/Food_UseSimple3.cfm

   to obtain a listing of containers that he/she may discard.

   b. Where possible, the test substance concentrate and containers should be returned to the registrant or manufacturer. Transportation must be according to all federal, state, and local laws and regulations.

   c. Follow label directions for use or disposal of the test substance if

option 2.b. is not available.

d. If no label directions exist for disposal, arrangements should be made with a licensed waste disposal firm for pickup and disposal of the test substance and/or the empty containers.

3. Disposal of test substance rinse water, unused spray solutions and other dilute test substance waste.

a. Check State and local laws and regulations to determine any procedures that may exist for proper disposal of test substance solutions.

b. Dispose of the dilute test substance waste in the field by adding to the spray tank and spraying on an overplanting of the crop where no contamination of plots will occur and where this procedure does not violate any laws or regulations. In the event that no overplanting exists, unused material may be placed along the field edge so long as contamination prevention measures are taken. All test substance solutions should be mixed with the intent of limiting excess amounts of solutions. If excess spray material is applied to an overplanting of crop, this area will be marked as treated and consumption prohibited.

SOP: 10.1, revision 14. Disposal of test substances.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 11.1 **Revision Number:** 12

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Liwei Gu **Date:** 1-29-2018

Title: Safety and health procedures in handling test substances.

Purpose: To assure that personnel handling test substances are doing so in a safe manner and if an accident occurs, danger is minimized.

Scope: All locations conducting field trials (including greenhouse).

Procedures:

1. Where institutional policies and guidelines do not exist, the following procedures should be followed.

2. All personal protective equipment and clothing as required by the label or written SOPs should be worn in the handling of test substances for storage, mixing and application. Emergency personal protective equipment (e.g. coveralls, self-contained breathing apparatus) must be available when handling hazardous test substances such as restricted use pesticides.

3. Appropriate weather conditions for the application of the test substance should prevail. Schedule application for desirable conditions, if possible.

4. All precautions should be taken to avoid applying test substances to or near sensitive areas or where drift to these areas may occur.

5. Prior to application, the equipment should be checked to make sure there are no leaks in the pump or tanks, hose connections, or worn spots in the hoses. All spray tanks should have lids. Filling the spray tank should be done carefully so it does not run over. All machinery should be shut down if it is necessary to adjust or repair any moving parts. Never blow out nozzles, hoses, or clogged lines by mouth. Inspect all test substance containers for leaks, tears, or holes before handling. Do not mishandle or abuse containers and thereby create hazards and/or emergencies by carelessness.

6. All test substances should be mixed in quantities which are adequate for the

job to avoid excess dilute solutions after the job is completed. Cleanup procedures should be established whereby excess sprays can be safely discarded; preferably by spraying the material on an overplanting of the commodity, if labeled. In the event that no overplanting exists, unused material may be placed along the field edge so long as contamination prevention measures are taken. If excess spray material is applied to an overplanting of crop, this area will be marked as treated and consumption prohibited. The equipment should be washed off both inside and outside and all test substances and test substance containers should be returned to storage as soon after use.

7. At the end of the working day employees who have applied or mixed test substances should take a shower and change clothes. Clothing should be washed after the end of the day. In no case should the same clothing be worn on a second day after it has been worn during a test substance application.

8. Treated areas should not be entered until adequate time, as specified by information on the test substance, has elapsed. For persons who regularly handle organophosphates and/or large quantities of carbamates, a cholinesterase level should be determined at least monthly throughout the pesticide application season.

9. Do not permit unauthorized persons in the test substance storage area.

10. Do not store test substances next to food, feed, seed, fertilizer, or other articles intended for consumption or use by humans or animals. Do not store food, beverages, tobacco, clothing, eating utensils, or smoking equipment in any area where test substances are present.

11. Do not drink, eat food, apply cosmetics, or use tobacco in areas where test substances are present.

12. Wear protective gloves while handling containers and mixing or measuring test substances.

13. Do not put fingers in mouth or rub eyes while working with test substances. Personnel should avoid touching moustache, if applicable, to avoid inhalation of compound(s).

14. Wash hand thoroughly with soap and water immediately after handling test substances and, especially before eating, smoking, or using the toilet.

15. Treated areas should be posted with warning signs if required by label.

16. Test substance storage areas should be properly ventilated.

SOP: 11.1, revision 12. Safety and health procedures in handling test substances.

Reviewed By:	Date:

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 12.1 **Revision Number:** 9

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linda Culpepper **Date:** 1-29-2018

Title: Procedures to follow prior to an EPA inspection.

Purpose: To provide guidance to study personnel in responding to a request for an EPA audit or review by Office of Compliance Monitoring (OCM).

Scope: All locations conducting field trials under good laboratory practices (GLPs).

Procedures:

1. Notify the study director, quality assurance officer, and other interested personnel of the pending audit or review as soon as possible.

2. Arrange to have available the personnel who may be associated with the trials and/or facility audit.

3. Make sure someone who is authorized to accept the Notice of Inspection is going to be present at the start and finish of the inspection.

4. Prepare personnel for the inspection.

    a. Review position descriptions with technical personnel so they understand and can explain their role in the trial(s).

    b. Discuss possible questions that may likely come up about the trials or facility and make sure every one understands what to expect.

    c. Request personnel to answer the questions only to the extent needed and not to provide extraneous information. Do not volunteer information; keep answers direct and to the point.

    d. Make certain that technical personnel know the safety precautions needed for the work area.

    e. Be certain that all documents pertaining to the inspection are available. This would include:

1.) Master schedules for Field Research Director (FRD).

2.) Study protocol and standard operating procedures (SOPs).

3.) Raw data, correspondence and logs.

4.) Training records, C.V.s, job descriptions, etc. of personnel.

5.) Appropriate chain of custody documents for samples and freezer logs and storage temperature documentation.

6.) Documentation of the characterization of the test substance, receipt, and handling, and storage records.

7.) Calibration logs on equipment such as balances and application equipment.

5. Have accessible organizational charts, a map of the facility and any information specific to the facility or area that will make the inspection go smoothly.

SOP: 12.1, revision 9. Procedures to follow prior to an EPA inspection.

Reviewed By:	**Date:**

N. C. State University, IR-4 Field Research Center
Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 12.2 **Revision Number:** 8

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Gibbs **Date:** 1-29-2018

Title: Procedures to follow during an EPA inspection.

Purpose: To provide guidance to study personnel in responding to a request for an EPA audit or review by Office of Compliance Monitoring (OCM).

Scope: All locations conducting field trials

Procedures:

1. Greet the inspection team and follow any institutional procedures for signing in. Provide name tags and escort the entire group to a conference or meeting room.

2. At the opening of the conference ask the lead inspector for his credentials and for any opening statements.

3. Introduce the facility personnel present and state their function in the facility or trials. Identify the person responsible who will accept the Notice of Inspection.

4. Distribute organizational charts, map of the facility and any other information previously prepared to make the inspection go smoothly.

5. Ask the lead inspector for his/her agenda for the inspection as well as a list, if possible, of personnel for interview during the inspection.

6. Explain any housekeeping rules such as the use of safety equipment in work areas etc. to avoid any possible misunderstandings.

7. Proceed with the inspection.

    a. Provide documents requested and provide explanations needed.

    b. Keep notes of observations and of all interviews.

    c. Keep management informed of the progress of the inspection and

the findings.

SOP: 12.2, revision 8. Procedures to follow during an EPA inspection.

Reviewed By:	Date:

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Room 110, 520 Brickhaven Dr.
Raleigh, NC 27606

Effective Date: Date of Approval

SOP#: 12.3 **Revision Number:** 8

Submitted by: Roger B. Batts RBB **Date:** 1-9-18

Approved by: Linei Gu **Date:** 1-29-2018

Title: Procedures to follow after an EPA inspection.

Purpose: To provide guidance to study personnel in responding to a request for an EPA audit or review by Office of Compliance Monitoring (OCM).

Scope: All locations conducting field trials.

Procedures:

1. Make sure that all personnel involved in the inspection are present for the closeout conference.
2. Clarify any discrepancies brought up during the exit interview and offer supporting documentation for clarification.
3. If you have corrected any problems during the inspection make sure the corrections are so noted in the inspector's logbook
4. Have someone present during the close-out take accurate notes.
5. Be sure you obtain a copy of the list of documents or other materials that may be taken as exhibits by the inspectors.
6. Debrief management, staff, and the study director with an explanation of any problems found.
7. Assign responsibility for preparation of possible solutions to problems and obtain time estimates for implementation.
8. Prepare any replies to the regulatory agency as necessary within a timely basis and keep interested parties such as management and the study director informed.

SOP: 12.3, revision 8. Procedures to follow after an EPA inspection.

Reviewed By:	Date:

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Appendix

Determining Nematode Population Responses to Control Agents

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Chemical control agents are necessary for the efficient production of many crops. If nematicides are to be tested and used properly, there must be an informed consensus on reliable, standardized sampling procedures and extraction methods for the target nematodes. Regardless of the methods used to evaluate nematode responses to nematicides, any weaknesses in the experiments will be reflected in the results.

In evaluating the efficacy of nematode control agents, characterizing nematode population responses is as important as determining plant responses. Most nematicides exert their maximum effect on nematodes shortly after application, but long-term changes in soil biology often occur. For example, the proportions of species in nematode communities often shift after chemical treatments of soil. In addition, some nematicides may stimulate plant growth whether or not nematodes are present; others affect insect, fungal, or bacterial populations or a combination of these.

The most important considerations in determining nematode population responses to control agents are collection of representative soil or root samples or both from each plot, adequate mixing of these samples, and extraction of nematodes by the most appropriate procedure for the target species and soil type. Such procedures depend on the kinds and numbers of nematodes present, their characteristics, and the nature and condition of the samples, including soil texture and time of collection (Tables 1 and 2). If any of these procedures is to be used reliably to evaluate responses to nematode control agents, investigators must understand the population dynamics of the target nematode on the particular crop and the specific geographic region involved. Where nematode numbers are below a detectable level, an appropriate bioassay should be used.

Because no single method of collecting nematode samples for assay or extracting nematodes to evaluate

Revision of a 1978 paper prepared by the Joint SON/ASTM E35.16 Task Force on Nematode Population Response, K. R. Barker, chairman.

Table 1. Extraction procedures for combinations of nematodes and soil types[a]

	Type of nematode (and soil type)											
	Meloidogyne spp.[b]			*Globodera* and *Heterodera* spp.[c]			Endoparasites[d]			Ectoparasites[e]		
Extraction technique	Sandy soil	Clay soil	Organic soil	Sandy soil	Clay soil	Organic soil	Sandy soil	Clay soil	Organic soil	Sandy soil	Clay soil	Organic soil
Baermann trays (31)	X	X	X	X	X	X	X	X	X	X	X	X
Blender and Baermann trays (31)							X	X	X			
Cobb's decanting-sieving and Baermann trays (31)	X			X			X	X		X	X	
Centrifugal flotation (16)	X	X	X	X	X	X	X	X	X	X	X	X
Centrifugal flotation with heavy sugar (9)				X	X	X						
Elutriation and Baermann trays (4)	X	X		X	X		X	X		X	X	
Elutriation and centrifugal flotation (4)	X	X		X	X		X	X		X	X	
Shaker extraction (3)							X	X	X			
Elutriation and dissolution egg masses (4,5)	X	X	X									
Elutriation (flotation) and dissolution cysts (1,4,5)				X	X							
Fenwick or flotation can (1,27)				X	X	X						
Mist chamber (24)							X	X	X			
Sugar-flotation sieving (7)	X						X			X		

[a] See Table 2 for selection of specific techniques for given sampling times.
[b] Also for *Nacobbus*, *Rotylenchulus*, and *Tylenchulus* spp.
[c] If hatching factor is required, use Fenwick or flotation can only.
[d] *Pratylenchus*, *Radopholus*, *Hoplolaimus*; for some plants, *Helicotylenchus*, *Tylenchorhynchus*, *Ditylenchus*, and *Aphelenchoides*.
[e] *Belonolaimus*, *Dolichodorus*, *Helicotylenchus*, *Hemicycliophora*, *Longidorus*, *Paratylenchus*, *Rotylenchus*, *Scutellonema*, *Paratrichodorus*, *Tylenchorhynchus*, and *Xiphinema*. Sugar-flotation sieving is most efficient for large forms such as *Belonolaimus* and *Xiphinema*. Researchers should use centrifugal flotation or elutriation and centrifugation for *Criconemella* spp.

nematicides can be used in all situations, the procedures described in this chapter are presented only as guidelines. However, specific procedures for sampling and extracting nematodes are recommended for various combinations of crops, soil textures, sampling periods, and nematodes. The last section is a glossary of some useful descriptive terms.

SAMPLING

Sampling procedures vary, depending on the crop, nematode species, host-parasite relationships, and soil type. Ideal times for sampling (when differences in population densities between treated and untreated plots are greatest) also vary with the crop, nematode, and geographic location.

The primary objective in sampling is to collect a sample that truly represents the population in a given plot or field at a given time. The spatial distribution of nematode populations varies greatly both horizontally and vertically. Most species occur as aggregates (egg masses or cysts) in a clustered (contagious) distribution (2,13) rather than in either a uniform or a random pattern (Fig. 1). The populations also may be skewed in distribution because of plant or soil influences. For these reasons, sampling is frequently the weakest link in field evaluations of nematicides (Fig. 2).

Important considerations in soil sampling include the number, diameter, and depth of cores needed to provide

Table 2. Extraction procedures for use at various sampling times for nematode parasites of annual and perennial crops[a]

	Type of nematode (and sampling time)[b]																			
	Meloidogyne spp.[c]				*Globodera* and *Heterodera* spp.[d]				*Criconemella* spp.[e]				Endoparasites[f]				Ectoparasites[g]			
Extraction technique	Pi	Pie	Pm	Pf	Pi	Pie	Pm	Pf	Pi	Pie	Pm	Pf	Pi	Pie	Pm	Pf	Pi	Pie	Pm	Pf
Annuals																				
Baermann trays (31)	X	X	X		X	X	X	X					X	X	X		X	X	X	X
Bioassay-gall index (19)			X(?)	X																
Blender and Baermann trays (31)													X		X	X				
Cobb's decanting-sieving and Baermann trays (31)	X	X	X	X	X	X	X	X					X	X	X	X	X	X	X	X
Centrifugal flotation (16)	X		X(?)	X			X(?)	X	X	X[e]	X[e]	X	X		X(?)	X	X		X(?)	X
Centrifugal flotation with heavy sugar (9)					X			X												
Elutriation and Baermann trays (4)	X	X	X	X	X	X	X	X					X	X	X	X	X	X	X	X
Elutriation and centrifugal flotation (4)	X		X		X			X	X	X[e]	X[e]	X	X			X	X		X(?)	X
Elutriation and dissolution egg masses (4,5)			X	X																
Elutriation (flotation) and dissolution cysts (1,4,5)					X		X(?)	X												
Fenwick or flotation can (1,27)					X		X(?)	X							X	X				
Mist chamber (24)															X	X				
Shaker extraction (3)													X		X	X	X		X	X
Sugar-flotation sieving (7)	X									X[e]	X[e]									
Vital stain (22)																				
Perennials																				
Baermann trays	X	X	X	X									X	X	X	X	X	X	X	X
Bioassay-gall index	X		X	X									X	X	X	X				
Blender and Baermann trays																				
Cobb's decanting-sieving and Baermann trays	X	X		X									X	X	X	X	X	X	X	X
Centrifugal flotation	X		X(?)	X					X	X[e]	X[e]	X	X	X	X	X	X	X	X	X
Centrifugal flotation with heavy sugar					X		X	X												
Elutriation and Baermann trays	X	X	X	X	X	X	X	X					X	X	X	X	X	X	X	X
Elutriation and centrifugal flotation	X		X(?)	X	X		X	X	X	X[e]	X[e]	X	X		X(?)	X	X	X	X	X
Elutriation and dissolution egg masses	X		X	X																
Elutriation and dissolution cysts					X		X	X												
Fenwick or flotation can					X		X	X												
Mist chamber													X		X	X				
Shaker extraction													X		X	X				
Sugar-flotation sieving	X		X	X									X		X	X	X		X	X
Vital stain										X[e]	X[e]									

[a] See Table 1 for consideration of soil type, Table 3 for data required for sampling times.
[b] Sampling times: Pi = initial nematode density, Pie = posttreatment nematode density, Pm = midseason nematode density, and Pf = final nematode density.
[c] Also for *Nacobbus*, *Rotylenchulus*, and *Tylenchulus* spp.
[d] If hatching factor is required, use Fenwick or flotation can only.
[e] Need vital stain to identify living specimens for Pie and sometimes Pm sampling.
[f] *Pratylenchus*, *Radopholus*, *Hoplolaimus*; for some plants, *Helicotylenchus*, *Tylenchorhynchus*, *Ditylenchus*, and *Aphelenchoides*.
[g] *Belonolaimus*, *Dolichodorus*, *Helicotylenchus*, *Hemicycliophora*, *Longidorus*, *Paratylenchus*, *Rotylenchus*, *Scutellonema*, *Paratrichodorus*, *Tylenchorhynchus*, and *Xiphinema*. (Sugar-flotation sieving is most efficient for large forms such as *Belonolaimus* and *Xiphinema*.)

A					
50	100	0	0	0	0
100	400	0	0	0	0
0	0	0	125	100	0
0	0	0	0	50	0
0	50	0	0	0	0
125	275	0	0	0	335

B					
40	45	50	55	45	50
45	55	45	40	40	50
50	45	45	45	50	55
45	50	55	45	50	45
55	50	40	50	45	45
50	40	50	50	55	40

C					
30	30	47	38	30	43
27	45	60	52	101	64
36	30	60	50	25	53
24	21	68	61	50	0
71	52	76	45	74	67
50	43	30	50	60	47

Fig. 1. Theoretical distribution patterns of nematode populations. **A**, Aggregate or contagious ($o^2 > \bar{x}$; $\bar{x} = 47.5$, $o^2 = 9{,}316$, $\Sigma = 1{,}710$). **B**, Uniform ($o^2 < \bar{x}$; $\bar{x} = 47.5$, $o^2 = 22.9$, $\Sigma = 1{,}710$). **C**, Random ($o^2 = \bar{x}$; $\bar{x} = 47.5$, $o^2 = 360$, $\Sigma = 1{,}710$; although $o^2 > \bar{x}$ in this case, the population distribution is more random than the example in **A**).

an adequate sample; the choice of a sampling pattern that will give reliable, representative data on population densities; the choice of sampling times that will reflect population density at critical stages during the growing season; the condition of the soil; and the proper handling and storage of samples.

Soil should be moist but not wet, preferably below 60-cm tension (percentage) of water suction (field capacity) for most sampling. The percentage of moisture should be determined from three to five representative samples per sampling time. Before extraction for cysts of *Heterodera* or *Globodera* spp., certain clay or silt soils (which become hard when dry) may be dried slowly and crumbled daily during the drying process.

Four samplings should be made to evaluate fully the effects of chemical soil treatments on nematode populations and plant growth: a pretreatment sampling (within 1 week before treatment), an intermediate posttreatment sampling (2–4 weeks after treatment), a midseason sampling, and a final sampling near or at harvest or the end of the growing season. In each case, the nematode population density should be determined per 100–500 cm^3 of soil. Nematicide treatments are often applied in the fall to avoid excessive delays in spring planting in northern areas. In such situations, sampling schedules need to be modified accordingly (the intermediate posttreatment sampling would be in the spring). Posttreatment samples collected in late fall and early spring are also necessary to determine nematicide effects on perennials.

The ideal sampling times may be identified more precisely by experimentally determining the degree-hours or -days (11) or the cumulative index of activity (17) required for the maximum population increase. The application of degree-hours (or -days) is helpful except where extremes of temperatures and/or moisture are encountered. A comparable model that relates temperature to the development (D) of root endoparasites, offered by Jones (17), is as follows:

$$D = k \sum_{1}^{n} (T_n - T_b),$$

where k is a temperature coefficient, T_n is the mean daily temperature, T_b is the basal temperature below which

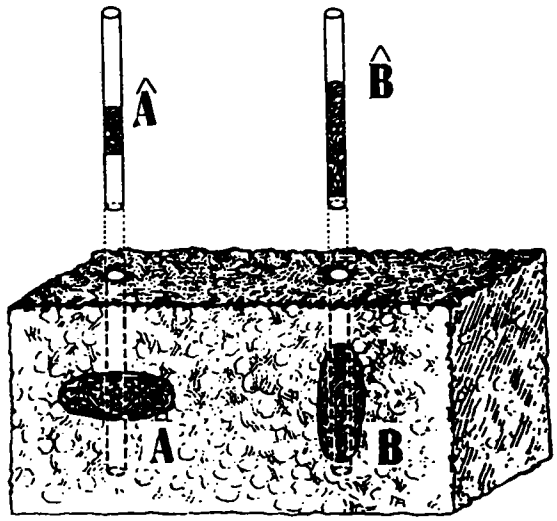

Fig. 2. Soil profile containing two aggregates of nematodes of equal population density (A = B/100 cm^3 of soil). Because of the difference in the axis orientation, the population densities estimated from soil cores are vastly different ($\hat{A} \sim 0.3\,\hat{B}$/100 cm^3 of soil).

growth is essentially zero, and $T_n - T_b$ is the effective temperature that is survived for n days. Jones developed a similar model, based on rainfall, for ectoparasites. His concept of combining cumulative temperature and rainfall for calculating an "index of activity" for nematodes may eventually be helpful in developing sampling schedules.

To standardize sampling procedures, researchers should use a sampling tube 2.0 cm in inner diameter (ID) or a cone-shaped tube (14) and sample to a depth of 20 cm unless otherwise indicated. Cores should be taken to a depth of 30–45 cm for the pretreatment sampling in regions with hot, dry summers; in such situations, a hydraulic sampling device may be necessary. Each core (2.0 cm in diameter by 20 cm deep) provides about 62 cm^3 of soil. Enough soil cores should be collected in a systematic procedure (Fig. 3A) to cover each plot according to its size as follows: small plots (1–5 m^2), 10 cores; medium-size plots (5–100 m^2), 20 or more cores, and large plots (greater than 100 m^2), 30 or more cores.

The pretreatment distribution pattern of target nematodes should be determined on a quadrat basis

when a high degree of precision is required. The actual distribution patterns can be characterized by a number of statistical procedures and parameters. The negative binomial distribution, described by two parameters, the mean ($\bar{x}$) and the dispersion parameter (k), can be fitted to nematode sample data from clustered distributions (2,13). The distribution approaches that of the Poisson as k approaches infinity (for practical purposes, $k \geq 8$ indicates a population with a near-random distribution). Values of k less than 1.0 indicate that the distribution is approaching the logarithmic series that occurs when $k = 0$ (10,28).

A relatively simple equation for estimating k is:

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}},$$

where $\bar{x}$ is the population mean and s^2 is the sample population variance. Fitting the negative binomial distribution to population data and estimating k can be done by available computer programs and more complex models (10,18).

The mean and k parameters may be used as a basis for estimating the number of samples needed for given degrees of precision (18). The number of cores needed for clustered populations, which can be described by the negative binomal distribution, may be determined as follows (18):

$$n = (Z\alpha/2)^2 \frac{(1/\bar{x} + 1/k)}{E^2},$$

where n is the number of samples, Z is the upper $\alpha/2$ point of the standard normal distribution, $\bar{x}$ is the population mean, k is the experimentally derived dispersion parameter of the negative binomial distribution, and E is the predetermined standard error as a decimal of the mean. This model, described by Karandinos (18), is more rigorous than those given by other authors (2,28) but is useful for single-composite sample estimations of populations. The simple model

$$n = \frac{(1/\bar{x} + 1/k)}{E^2}$$

given by Southwood (28) should be adequate for determining nematicide efficacy in small-plot tests.

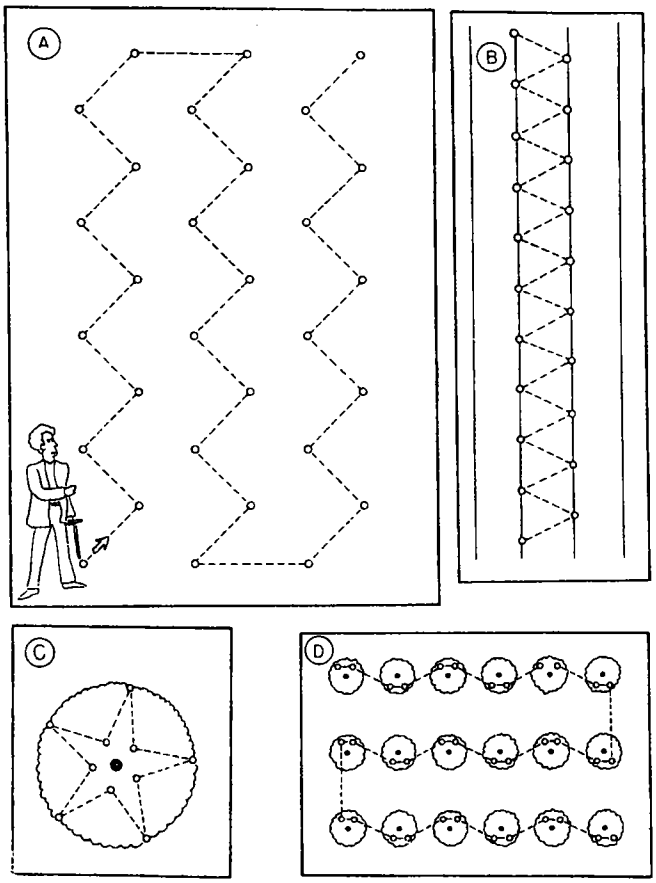

Fig. 3. Schemes for collecting soil samples. A, Recommended pattern for collecting a minimum of 20–30 cores for pretreatment nematode assay of large test field or plot. B, Sampling pattern for collecting soil from two center rows in four-row plot (performance is determined only from the two center rows). C, Procedure for sampling single-plant plot. D, Pattern for sampling in feeder-root zone of established perennials (for small plots, additional cores should be collected from each plant).

Careful collection of soil samples by well-trained, responsible individuals is of utmost importance for reliable nematode assays. The sampling procedure, however, must be modified for different kinds of crops. With established annual row crops, sampling should be done in the row, with cores coming from the root zone, 5–12 cm from the stems (Fig. 3B). Special care must be given to single-plant plots (Fig. 3C). For deep-rooted perennials such as grape and citrus, samples should be collected at two or three depths (15, 30, and 60–100 cm). Sampling to a depth of 8–12 cm is sufficient for shallow-rooted perennials such as turfgrasses. For ornamentals and other perennials such as fruit trees, borings should be collected in the drip line to a depth of 20 cm (Fig. 3D). Samples should be taken at several depths down to 90 cm for some fruit trees on deep alluvial soils.

Extracting nematodes from roots and soil at mid-season and final samplings is often necessary for certain endoparasites such as *Pratylenchus* spp. (Tables 2 and 3). A larger sampling tube (5.0 cm ID) should be used to obtain roots with such soil samples. Alternatively, the researcher may dig and collect root samples from a minimum of 10 plants per plot.

Initial Nematode Density

Each plot should be sampled in a systematic manner within 1 week before treatment. This sampling is particularly important to establish a base population density for each individual plot, because nematode populations may vary greatly from plot to plot. These base population densities allow more precise estimation of the effects of various nematicides in each plot.

Posttreatment Nematode Density

The intermediate posttreatment sampling can be used to relate numbers of nematodes to crop performance for fast-acting nematicides (fumigants).

Caution: Do not collect soil treated with highly toxic, slow-acting materials such as organic phosphates or carbamates within 14 days after application.

Fumigant nematicides. With fumigant nematicides, plots should be sampled 2–3 weeks after application in the same pattern as the initial sampling if the treatment is broadcast. For row treatment, the sampling should be done in the row.

Table 3. Nematode population data required from standardized-volume soil samples from annual and perennial crops[a]

Sampling time[b]	Type of nematode: Sedentary forms — *Meloidogyne* spp.	Sedentary forms — *Globodera* and *Heterodera* spp.	Sedentary forms — *Criconemella* spp.	Migratory forms — Endoparasites	Migratory forms — Ectoparasites
			Annuals		
Pi	Juveniles in soil	Cysts, eggs, and juveniles in soil	Nemas in soil	Nemas in soil	Nemas in soil
Pie	Living juveniles in soil	Living juveniles	Living nemas in soil	Living nemas in soil	Living nemas in soil
Pm	Juveniles and eggs (soil and roots)	Eggs, juveniles, and cysts	Living nemas in soil	Living nemas in soil and roots	Nemas living in soil
Pf	Above plus gall index	As above	Nemas in soil	Nemas in soil and roots	Nemas in soil
			Perennials		
Pi	Juveniles and eggs (soil and roots)	—	Nemas in soil	Nemas in soil and roots	Nemas in soil
Pie	Living juveniles	—	Living nemas in soil	Living nemas in soil and roots	Living nemas in soil
Pm and Pf	Same as for annuals				

[a] See Tables 1 and 2 for specific extraction procedures for specific combinations of types of nematodes and soil.
[b] Sampling times: Pi = initial nematode density, Pie = posttreatment nematode density, Pm = midseason nematode density, and Pf = final nematode density.

Table 4. Nomograph of root-knot galling indexes for *Meloidogyne* spp.

Percentage of root system galled	Galling index system[a] 0–4	0–5	1–6[b]	0–10
0	0	0	1	0
10		1	2	1
20		2	3	2
	1			
30				3
40				4
50	2		4	5
		3		
60				6
70				7
	3			
80		4	5	8
90				9
100	4	5	6	10

[a] Roots are scored for degree of galling using one of several root-galling indexes, all of which are comparable and relatively interchangeable. A minimum of 10 root systems should be evaluated per treatment.
[b] We recommend this scheme for use in evaluating nematicides (see Fig. 5 for examples similar to those of Zeck [34]).

Nonfumigant nematicides. Sampling should be done 4–6 weeks after application, because most of these compounds are slow-acting. Extraction methods that depend on nematode motility may be used, or a vital stain (22) may be used to recognize living nematodes at this sampling time.

Midseason Nematode Density

The optimum time for the critical midseason sampling for determining the efficacy of nematicides varies with the life cycle of the target nematode species, host-parasite relationships, crop, climate, and type of nematicide. Normally it is best done when the population density in untreated controls is high (6–12 weeks after treatment). If too much time elapses after treatment, nematode numbers in treated plots often exceed those in controls because of the larger root system and lack of competitors or predators or both in the treated plots. As mentioned earlier, sampling may be based on the physiologic time (cumulative heat units above the activity threshold [11]) required to complete a life cycle. When this information is available, the midseason sampling particularly should be scheduled accordingly. For many perennials, e.g. turfgrasses, or when little is known about the population dynamics of the target species, sampling monthly for 5 or 6 months is desirable. Final population densities are often more useful than midseason densities in northern geographic regions with short growing seasons.

Final Nematode Density

The final population is usually determined at the time of harvest for annual crops. This sampling is most useful for determining residual effects of nematicides. Nematode numbers in treated plots often exceed those in untreated plots at this sampling. For *Meloidogyne* spp., root-knot indexes should be obtained at this time (Table 4).

CARE AND CONDITIONING OF SOIL SAMPLES

Soil samples for nematode assays should be regarded as perishable and handled accordingly. Exposure to temperatures of 40°C or above, even for a short time, kills some species. All soil and root samples should be placed in plastic bags to prevent drying, kept out of the sun, and transported in an insulated container.

Ideally, nematodes should be extracted no later than 2 days after soil samples are collected; however, longer storage is often necessary. Samples should be stored at 10–15°C to keep the nematodes physiologically young and active. Lower storage temperatures may be desirable in northern regions. High temperatures allow hatching of eggs and rapid aging of motile forms; low temperatures may cause chilling injury.

Adequate mixing of composite soil samples before an aliquant (usually 100 cm^3) is removed for extracting nematodes is also important when the entire sample (500 cm^3 or more) cannot be processed. Such samples should be screened first and then mixed thoroughly by a

suitable procedure, e.g., running them through a soil sample splitter two to four times. Also, soil samples may be mixed effectively by coning and quartering. Mixing the composite sample avoids undue variation caused by nematode aggregation. Root or other plant tissues should be cut and mixed when an aliquant is selected for extraction. Chopping tissues in blenders may be satisfactory in some instances but is not recommended for general use (because of toxic substances released by some plants, such as peach).

Caution: Although mixing soil is necessary, special care (gentle mixing or use of flotation procedures) must be given to samples containing *Xiphinema, Longidorus*, or *Paratrichodorus* spp., which are often injured and lose their motility by mechanical mixing of soil.

CALIBRATION AND STANDARDIZATION

Concentrations of all chemicals used for extracting nematodes should be expressed on a molar basis—micrograms per milliliter or gram (see specific procedures). In procedures using the centrifuge, the relative force times gravity (*g*) should be determined rather than simply the number of revolutions per minute (rpm). When stirrers are used, rpm is sufficient. Subsamples should be based on volume of soil rather than weight, because weight varies greatly with soil moisture. The water content should be determined and reported as a percentage of oven-dry weight of soil.

SAFETY PRECAUTIONS

Certain hazards are associated with some of the methods described. When using the NaOCl procedure for extracting eggs of *Meloidogyne* spp. (5) and other nematodes that produce external egg masses or for dissolving cysts to free eggs of *Heterodera* or *Globodera* spp., workers should use a fume hood to avoid inhaling the vapors. Soil samples should not be collected within 14 days after application of highly toxic nematicides (organic phosphates or carbamates). When soil assays are made within 2–4 weeks after applying chemicals at any residual concentration, appropriate precautions (protective gloves) should be taken in handling and mixing soil.

EXTRACTION METHODS

Knowledge of the biology and population dynamics of various nematode species is essential for selecting the most appropriate extraction method for each sampling time (Tables 1–3). Root samples are best used for endoparasites (such as *Meloidogyne* and *Pratylenchus*) and semiendoparasites (such as *Hoplolaimus* and *Helicotylenchus*) in evaluating chemical soil treatments, because a significant portion of these nematode populations may exist in the roots. Root sampling may not be necessary shortly after a chemical treatment, because the fractions of the populations in the soil reflect the relative efficacy of the test material. Many sedentary (immobile) ectoparasites can be extracted only by flotation procedures. For soil samples collected within 1–3 weeks after chemical soil treatments, methods that yield only motile nematodes are best. Vital stains (Phloxine B and new Blue R) may be used with procedures such as the centrifugal flotation method, which yields dead as well as live specimens (22). Many nonfumigant nematicides act over a period of 6 weeks or more, causing nematode starvation and slow disappearance from the soil (15). The timing of early posttreatment sampling must be adjusted for such materials.

Several factors may affect the efficiency of specific extraction procedures. Certain problems, such as losing nematodes through sieve openings, occur with numerous techniques. Procedures and potential major difficulties are listed in Table 5.

Selection of assay procedures depends on the kinds and numbers of nematodes present, host, nature and condition of the samples (including soil texture), time of collection, and chemical soil treatments used. Nematode populations may consist of relatively motile to quiescent forms—cysts, individual eggs, eggs in masses, or various combinations of these. Ectoparasitic forms occur primarily in the soil, whereas high percentages of endoparasites are in roots, root fragments, or other plant parts during certain periods of the year. Actively moving juveniles or adults (in roots or soil) can be

Table 5. Extraction procedures and potential major difficulties

Procedure	Difficulties
Baermann methods, sieving-Baermann trays	Excessive soil or debris; inappropriate temperature used for incubation; nematode motility; excessive microbial activity; excessive water over soil
Centrifugal flotation	Inappropriate type or use of centrifuge or sieves; high amount of organic matter; nematodes primarily in roots; incorrect sugar concentration
Sugar-flotation sieving	High moisture, especially with clay soils; high clay or organic content of soil; incorrect sugar or Separan concentration; incorrect mesh of sieves; dark color of molasses, which, if used in lieu of sugar, interferes with decanting
Elutriators	Incorrect rate of water flow; wrong sieve size; soil texture (high organic matter or clay)
Mist techniques	Evaporation in dry climates, which may reduce water temperature below optimum (22–25°C at root level) for some nematodes; toxic substances in roots (e.g., peach if chopped in blender)
Fenwick or flotation can	Excessive moisture in cysts; high organic matter
Shaker incubation	Excessive microbial activity; inappropriate incubation temperature (22–25°C optimum for nematode species)
Semiautomatic elutriator	Incorrect water, airflow, sieves, or all three; high organic matter
Elutriator-NaOCl egg extraction	Erratic root distribution and egg hatching before assay; incorrect concentration of NaOCl for dissolving cysts of *Heterodera* or *Globodera* spp.

extracted by Baermann trays or modifications thereof, a combination of flotation or sieving or both and Baermann methods, or flotation (or elutriation) and cotton wool methods. Methods adapted to both motile and nonmotile forms include sieving, centrifugal flotation, sugar-flotation sieving, and Seinhorst's two-Erlenmeyer-flask sedimentation apparatus. The flotation methods are of limited use for endoparasites when most are inside the roots or other plant parts, but they are superior for ectoparasites. For the extraction of migratory nematodes from roots, the Seinhorst mist apparatus is ideal, but incubation on a gyratory shaker (3) is also satisfactory. Methods for extracting free eggs and egg masses are now being developed. Extraction of cysts of *Heterodera* and *Globodera* spp. also requires special techniques, such as the Fenwick or flotation can or "heavy sugar" centrifugation. If population densities are below a detectable level, an appropriate bioassay may be used (e.g., Rutgers tomato for most *Meloidogyne* spp., Lee soybean for *Heterodera glycines*).

Specific outlines for each recommended method of extracting nematodes follow. Several methods that require special equipment, excessive labor inputs, or both are not described. As indicated earlier, many nematodes are lost during sieving in any procedure. Such losses often can be minimized by allowing the nematodes to settle out of suspension and decanting the excess water instead of sieving. A second passage of the suspension may be necessary when population densities are low. Required equipment and chemicals are listed for each of the following methods.

EXTRACTION OF NEMATODES IN SOIL ONLY

Centrifugal Flotation

Centrifugal flotation (16, modified) is an excellent method for routine assays and is the best procedure when *Criconemella* is the target genus.

Equipment: Centrifuge with horizontal (swinging bucket) head with 50-ml or larger tubes and operation to 420 *g*; mechanical stirrers; 35-, 325-, and 400-mesh sieves; 100-, 150-, and 1,000-ml beakers; soil sample splitters (W. S. Tyler Co., Mentor, OH 44060) or coarse sieves (for mixing).

Chemicals: Sucrose solution, 454 g, in sufficient water to make 1 L of solution (specific gravity 1.18 or 38.5% by weight).

Note: Commercial detergents may enhance recovery by this method (33).

Procedure:

1. Mix the soil.
2. Place a 100-cm^3 aliquant in a 1,000-ml beaker and add sufficient water to bring the total volume to 600 ml.
3. Stir for 20 sec and allow the soil to settle for 60 sec (maximum time of 20–30 sec is best for *Criconemella* spp.).
4. Decant onto a 40-mesh sieve over a 325-mesh sieve. Hold sieves at an angle of about 35–40° during all decanting processes to minimize the chance of small nematodes passing directly through the sieve.
5. Using a wash bottle, rinse the 40-mesh sieve while still over the 325-mesh sieve (excessive rinsing washes small nematodes through both sieves). For *Heterodera* and *Globodera* spp., add a 60-mesh sieve between the 40- and 325-mesh sieves to collect cysts.
6. Wash the debris and nematodes from the 325-mesh sieve into a 150-ml beaker.
7. Pour the washings (step 6) into 50-ml centrifuge tubes.
8. Place the tubes in the centrifuge (be sure to balance the tubes).
9. Centrifuge at 420 *g* for 5 min.
10. Decant water from the tubes (nematodes are in the soil pellet in the bottom of the tubes).
11. Refill centrifuge tubes with sucrose solution and mix with stirring rod or vibrator mixer.
12. Centrifuge for 30 sec at 420 *g* (nematodes remain suspended in sugar solution). Do not use the brake on certain centrifuges, because it may cause enough vibration to dislodge the pellet.
13. Decant the sugar solution-nematode suspension onto a 400-mesh sieve (pour slowly when recovering small nematodes).
14. Rinse the residue and nematodes from the 400-mesh sieve into a 150-ml beaker (about 20 ml of water, suitable for making counts).

Flotation-Sieving Method

This method (7) is well suited for extracting large nematodes such as *Xiphinema* spp. from sandy soils. It generally is less efficient than centrifugal flotation but is acceptable if centrifuges are not available.

Equipment: Mechanical stirrers; 35-, 325-, and 400-mesh sieves; 100-, 150-, and 1,000-ml beakers; wash bottles or mist hoses; soil sample splitter or sieves (for mixing).

Chemicals: Sucrose solution (0.7*M* or 22.0% by weight); Separan NP10 (Dow Chemical Company, Midland, MI 48640), final concentration of 12.5 μg/ml.

Note: Molasses can be used in lieu of sucrose (specific gravity 1.1) (23).

Procedure:

1. Mix soil.
2. Place a 100-cm^3 subsample in a 1,000-ml beaker and add sufficient 0.7*M* sucrose and Separan (12.5 μg/ml) solution to bring total volume to 500 ml.
3. Stir with a motorized stirrer for 20 sec.
4. Allow the soil to settle for about 2 min.
5. Decant the liquid onto a 40-mesh sieve over a 325-mesh sieve.
6. Rinse the 40-mesh sieve while it is still over the 325-mesh sieve.
7. Using a wash bottle, rinse the nematodes and debris from the 325-mesh sieve into a 150-ml beaker (about 50 ml of water).
8. Swirl the 150-ml beaker and allow the contents to settle for 5–10 sec).
9. Decant the nematode suspension onto a 400-mesh sieve and rinse the residue into a 150-ml beaker (about 20 ml of water).

Semiautomatic Elutriator

This approach (4) to nematode extraction includes an elutriator similar to Oostenbrink's (20) plus a sample splitter and sieve shaker. It may be used in combination with Baermann trays or centrifugal flotation.

Equipment: Simple elutriator, Oostenbrink type; aqueous sample splitter; water and air supplies; motorized sieve shaker; 10-, 24-, 40-, 60-, 400-, and 500-mesh sieves. (This method may be semiautomated with time clocks, etc. [4].)

Procedure:

1. Add 500 cm^3 of unmixed soil to the elutriator (with air and water flowing at desired rates).
2. Run the elutriator for 3 min, catching roots on the 40-mesh sieve over a sample splitter and "free" nematodes on the 400-mesh sieve on the motorized shaker. A 10- or 24-mesh sieve should be used rather than the 40-mesh sieve for large nematodes such as *Xiphinema* that might be trapped on a 40-mesh sieve.
3. Rinse the sieves.
4. For eggs of *Meloidogyne* spp., process the roots from the 40-mesh sieve by the NaOCl method (5). For *Pratylenchus* spp. and other migratory endoparasites, roots are trapped on the 40-mesh sieve and incubated in the mist chamber. Cysts of *Heterodera* or *Globodera* spp. may be collected on a 60-mesh sieve under 10- and 24-mesh sieves. The eggs from cysts may be extracted by the NaOCl method (5).

Any fraction (1/15, 1/5, etc.) of nematodes in the soil is collected on sieves on the shaker. *Criconemella* and related genera may be cleaned by centrifugation flotation and other species by Baermann methods, sugar-flotation sieving, or centrifugal flotation with 500-mesh sieves.

Cobb's Decanting and Sieving

The simple, modified version described below of Cobb's (31) sifting and gravity method is useful in extracting nematodes for inoculation purposes and routine assays when combined with Baermann methods. Addition of Separan eliminates the need for numerous sievings. Thorne (30) describes the original method, and Townshend (31) gives illustrations.

Equipment: 10-L pails; 10-, 25-, 50-, 100-, 200-, 325-, and 400-mesh sieves; 50-, 150-, 250-, and 500-ml beakers.

Chemicals: Separan with sucrose can be used to reduce the steps involved. A stirrer and 600-ml suspension with 100 cm^3 of soil should be used as in the description of sugar-flotation sieving.

Procedure (short, modified method):

1. Place 500 cm^3 of soil in a large pan or pail and cover well with water containing Separan (12.5 μg/ml). Thoroughly break all lumps, mix by hand, and allow to settle about 2 min.
2. Decant onto a 40-mesh sieve over a 325-mesh sieve.
3. Resuspend the original soil in water and repeat step 2 if maximum recovery is desired.
4. Combine the washings from steps 2 and 3, stir, and allow them to settle for 10 sec.
5. Decant through a 400-mesh sieve and rinse the residue (nematodes) into a clean beaker for counting (may place on Baermann tray for cleaner samples).

EXTRACTION OF MOTILE SPECIES

Baermann Trays

The Baermann trays procedure (32) is useful for extracting nematodes from small soil samples, root fragments, and debris coming from elutriators and for extracting juveniles of *Heterodera* and *Globodera* spp. (in the spring).

Equipment: Plastic screen (sieve-type) 17.5 cm in diameter (20-mesh) supported with legs 4 mm high or similar metal unit, wet-strength facial tissues, epoxy resin-coated aluminum pie pans 20 cm in diameter or small plastic salad bowls.

Procedure:

1. Mix the soil.
2. Place a 100-cm^3 aliquant of soil uniformly over the tissue, which is superimposed on a plastic or stainless-steel screen (do not use copper screens).
3. Place the screen with soil in a 20-cm pie pan and add water just to cover the soil.
4. Incubate the samples at 21–24°C (cover or stack to reduce evaporation). Add water as needed.
5. Collect the nematodes from the pans after 3 days. If dirty, clean by pouring through a 500-mesh sieve. For maximum recoveries, nematodes should be collected over a period of 1–14 days.

Baermann Trays Plus Elutriation or Sieving

The combination of Baermann trays with elutriation (or sieving), a modification of the Christie-Perry procedure (8), is still invaluable for extracting nematodes from soil and root fragments.

Equipment: 10-, 24-, and 400-mesh sieves; elutriator; screen supports; pie pans (stainless-steel or resin-treated) 15–20 cm in diameter; wet-strength facial tissues; milk filter or muslin filter.

Procedure:

1. Add an unmixed 500-cm^3 soil sample to the elutriator.
2. Collect the root and soil fractions from 10- (or 24-) and 400-mesh sieves, respectively (see section on the semiautomatic elutriator), or use the modified Cobb's decanting-sieving method.
3. Place these fractions on facial tissues supported on screen.
4. Carefully add enough water to just cover the residue. Add more water as needed with time.
5. Collect the nematodes after incubation at 21–24°C for 3 days (for maximum recoveries, collect them over a period of 10–14 days).

Others

The semiautomatic elutriator approach is described herein. The Seinhorst elutriator (25) or Oostenbrink elutriator (20) procedures may also be used to extract motile nematodes.

EXTRACTION OF CYSTS

The use of a reliable method for determining numbers of eggs in cysts, and egg masses if present, is essential for evaluating the efficacy of nematicides on *Heterodera* and *Globodera* spp. A Ten-Broeck homogenizer (27) is satisfactory for this purpose. The NaOCl (sodium hypochlorite) method described in the section Extraction of Nematode Eggs also gives good results in dissolving cysts and freeing the eggs for counts (although the researcher may need to use two to three times the concentration indicated for egg masses of *Meloidogyne* spp.).

Flotation Can

The flotation can method (1) is an alternative to the Fenwick can (see next subsection). The use of 9:1 ethanol-glycerin in lieu of acetone-carbon tetrachloride offers many advantages. In addition to being very safe,

the ethanol-glycerin solution can be filtered and reused.

Equipment: Flotation can and device for separating cysts from organic debris (Fig. 4), sieves (20- and 100-mesh), soil crusher (for "brick-like" soils), stirrers, Whatman no. 4 filter paper, general glassware.

Chemicals: Ethanol, glycerin, materials as described in the section Extraction of Nematode Eggs for extraction of eggs by NaOCl.

Procedure for extracting cysts from soil:

1. Place 500 cm^3 of well-mixed soil in flotation can. Add water to within 10 cm of spout and mix by hand.
2. Add water at the bottom of the flotation can so that the flow rate is sufficient to wash over floating and suspended material but not silt or sand particles. Continue for 2 min, catching the overflow on 20- and 100-mesh Tyler sieves (clay particles that are washed over will pass through both sieves).
3. Turn off water and stir sediment thoroughly.
4. Add water at the correct rate for a further 2 min, catching the overflow on the 20- and 100-mesh sieves.
5. Turn off water and pour the contents of the can through the sieves until just before the sediment starts to flow out. Do not pour the sediment onto the sieves.
6. Wash the debris on the 20-mesh sieve with a jet of water so that cysts are flushed onto the 100-mesh sieve.
7. Wash the contents of the 100-mesh sieve (the "flotsam") onto tissue paper supported by a wire gauze sieve 10 cm in diameter.
8. Leave to dry.

Procedure for separating cysts from organic debris:

1. Fold Whatman no. 4 filter paper (24 cm in diameter) and place in funnel in flask with side-arm attachment (Fig. 4). Close funnel tap and fill with ethanol-glycerin mixture (9:1, v/v) to 2 cm from surface.
2. If the "flotsam" has aggregated, gently crumble it to free the cysts from adhering debris without crushing them.
3. Pour the finely divided "flotsam" onto the ethanol-glycerin mixture in the funnel. Cysts move outward to the filter surface. The major portion of the debris sinks to the bottom of the funnel and should be stirred with a spatula to release trapped cysts.
4. When all of the floating portion has reached the filter paper, apply a partial vacuum to the flask through the side arm and open the funnel tap cautiously. The ethanol-glycerin mixture passes through into the flask and can be reused. The floating portion containing the cysts remains as a thin line around the filter paper.
5. Unfold the filter paper and place on a shallow watch glass or glass plate. Cysts are almost all on the outermost edge of the line and can be counted under a dissecting microscope.
6. After cysts have been counted, wash the line through the funnel into a beaker.

Procedure for releasing and counting eggs from cysts (basic method as outlined in the section Extraction of Nematode Eggs for eggs of *Meloidogyne* spp.):

1. Blend cysts in water-sodium hypochlorite (NaOCl 5.25%) mixture (1:1, v/v), using a tissue homogenizer for 30 sec (time will vary with type of homogenizer and condition of cysts).
2. Transfer blended material onto 500-mesh sieve and rinse with gentle flow to remove NaOCl.
3. Transfer material from the 500-mesh sieve into a

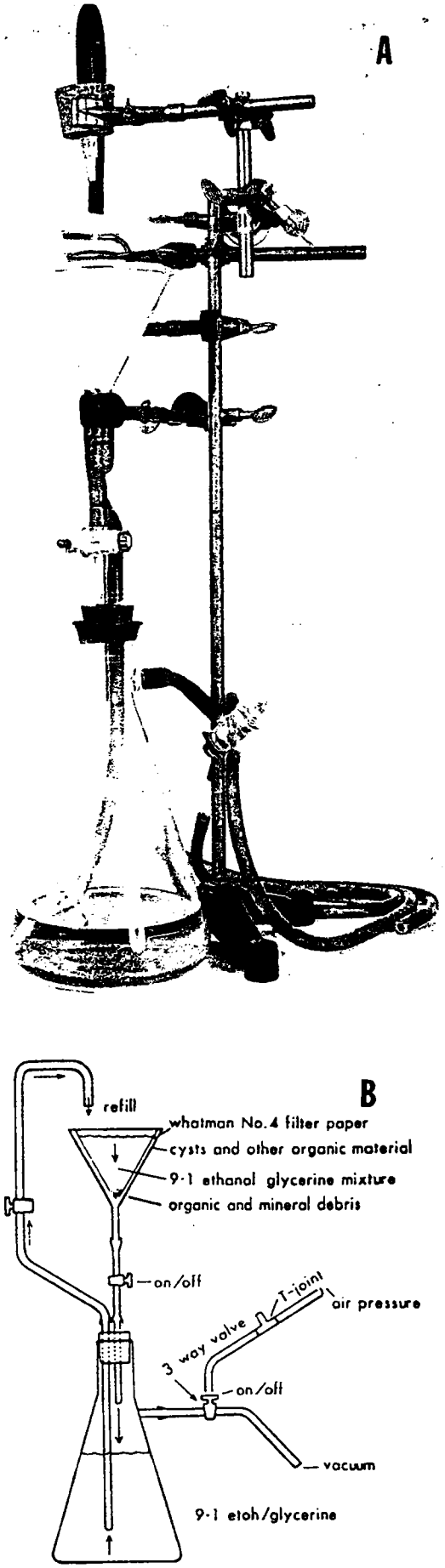

Fig. 4. A, Device for separating cysts of *Heterodera* or *Globodera* from organic debris. **B,** Diagram of the apparatus. (Courtesy Herb Quick)

150-ml beaker with fluted sides and make up to 100 ml with water (to 50 ml if low egg count is anticipated).

4. Stir with magnetic stirrer.

5. Remove 1-ml sample (5–10 ml for low egg count) with pipette into counting dish and count eggs and juveniles (do not count empty egg shells). (Staining eggs with acid fuchsin, as described in the section Extraction of Nematode Eggs for eggs of *Meloidogyne* spp. facilitates the egg counts.)

Flotation-Modified Fenwick Can

The flotation-modified Fenwick can procedure (27) is useful for extracting cysts from dry soil, but centrifugation with heavy sugar (described below) and other methods are becoming more widely used.

Equipment and chemicals: Modified Fenwick can, sieve (60–80 mesh) with bowl, camel's-hair brush (no. 00, 0, or 1), homogenizer (Ten-Broeck), plain glass slide, 100-ml bottle, pipette, aquarium pump, 400-ml Erlenmeyer flask, 250-ml volumetric flask, funnel, petri dish, filter papers 18.5 cm in diameter, acetone or acetone-carbon tetrachloride 3:1 (may use ethanol-glycerin [9:1] in lieu of acetone-carbon tetrachloride; see previous procedure).

Procedure:

1. Mix the soil thoroughly.

2. Fill the modified Fenwick can with water. Place the sample of 100 cm^3 of well-mixed soil in the top sieve (20.5 cm in diameter, 18- or 24-mesh).

3. Wash the sample into the apparatus via the funnel. The coarse material is retained on the top sieve, heavy soil particles such as sand sink to the bottom of the apparatus, and the floating cysts are carried off over the overflow collar.

4. Collect cysts, root debris, and other particles on a sieve 20 cm in diameter (60–80 mesh). Particles 175 μm and smaller pass with water through the sieve.

5. After washing, dry the debris at room temperature. Transfer the somewhat dried debris retained on the sieve to a 250-ml flask.

6. Pour technical acetone (or a mixture of 3 parts acetone and 1 part carbon tetrachloride) into a volumetric flask up to level 1 (neck of flask). Shake the flask and fill it completely. Be sure to use the exhaust hood.

7. After 1 min, decant the floating cysts and debris through a filter paper (18.5 cm in diameter) in a glass funnel into a second or Erlenmeyer flask while rotating the original flask. The acetone passes through the filter.

8. Place the filter in a petri dish and view it through a dissecting microscope (magnification ×50) with overhead light. Pick up the cysts with a camel's-hair brush (no. 00, 0, or 1) and transfer them to a small watch glass with moist filter paper. Identify the cysts under the dissecting microscope using an overhead light. Use a camel's-hair brush to transfer the cysts of the desired species into a small drop of water in the glass tube of the homogenizer. Place the piston in the tube and very carefully rotate it by hand. Pour the eggs and juveniles that were released from the cysts into a bottle. Fill the bottle with water up to 100 ml. Mix the suspension carefully using compressed air. Pipette out two 10-ml aliquants and place them in 10-ml Perspex dishes for counting. As indicated earlier, eggs should be freed by NaOCl or Ten-Broeck homogenizer and numbers determined as outlined in the subsection on the flotation can method.

Centrifugation with Heavy Sugar

This procedure (9, modified) is useful for isolating cysts and juveniles of *Heterodera* and *Globodera* spp., but problems may be encountered with some fine clay soils.

Equipment: Same as for centrifugal flotation for routine use, except that 24- and 100-mesh sieves are needed.

Chemicals: 1.8*M* (50% by weight) sucrose solution (615 g of sucrose) dissolved in enough warm water to make 1 L of solution (specific gravity 1.23).

Procedure:

1. Wash 100 cm^3 of soil through a 24-mesh sieve and collect it in a beaker (use about 1 L of water).

2. Mix the suspension thoroughly and allow it to settle for 10–15 sec.

3. Pour the supernatant through a 100-mesh screen (add a 325-mesh sieve for juveniles).

4. Wash any residue from the screen into a centrifuge tube or tubes with 1.8*M* sucrose solution.

5. Centrifuge at 420 *g* for 2.5 min.

6. Collect the supernatant on a 100-mesh screen (add a 325-mesh sieve for juveniles).

7. Rinse thoroughly.

8. Wash the sample into a beaker, using about 20 ml of water.

9. Crush the cysts with a Ten-Broeck homogenizer or dissolve them with NaOCl, as described for the flotation can method, and count the eggs and juveniles.

Others

The semiautomatic elutriator method (4) is useful for recovering cysts of *Globodera* and *Heterodera* (see previous description). Seinhorst's extraction procedure (25) may also be used for *Heterodera* and *Globodera* cysts from moist soil.

SEPARATION OF NEMATODES FROM PLANT TISSUES

Modified Seinhorst Mist Apparatus

This mist chamber method (24) is the most widely used method for obtaining nematodes from plant tissues.

Equipment: Time clock, water mixer-warmer, water regulator and filter, solenoid switch, cover (fiberglass, Plexiglas, stainless steel, or other suitable material) with doors to funnel racks or supports, superfine nozzles, plastic petri dishes, glass funnels 10 cm in diameter with rubber tubing, 1-L plastic cups with holes in the bottom, clamps, 500-mesh sieve (specifications available from K. R. Barker).

Procedure:

1. Place a representative sample of roots or other plant tissues in plastic cups superimposed over an open Baermann funnel. This funnel is supported over a second Baermann funnel by a plastic petri dish with a 2.5-cm hole in the center. Use wet-strength facial tissues in the cups to reduce debris.

2. Set the time clock to regulate desired mist (on 1 min, off 2 min). Adjust the water mixer to the flow rate that gives a temperature of 24°C.

3. Collect the nematodes from the funnels every 3–5 days through a 14-day period (nematodes such as *Pratylenchus* spp. will continue to emerge for weeks).

4. Concentrate and clean the nematode suspensions if necessary with a 500-mesh sieve.

Shaker

Several researchers have found that this shaker procedure (3) yields numbers of *Pratylenchus* spp. and other genera from roots similar to those obtained from mist chambers.

Equipment: Gyratory shaker, 125-ml flasks.

Chemicals: Ethoxyethyl mercuric chloride (Aretan), streptomycin sulfate or other suitable antibiotics.

Procedure:

1. Wash roots and cut into segments 1–2 cm long.
2. Place a representative sample of root tissue (0.5–5.0 g) in a 125-ml flask.
3. Cover the tissue with a mixture of 10 μg/ml of ethoxyethyl mercuric chloride and 50 μg/ml of streptomycin sulfate.
4. Incubate the mixture at 100 rpm for 48 hr.
5. Collect nematodes on a 325- or 400-mesh sieve; rinse them into a 150-ml beaker and count them.

Blender-Baermann Tray

Although many problems are encountered with this procedure (29), it is useful for limited situations.

Equipment: Blender, beakers, 325-mesh sieve, Baermann funnels or pans.

Chemicals: Ethoxyethyl mercuric chloride in a 10-μg/ml solution (other materials such as antibiotics or certain fungicides [captan] may be used instead).

Procedure:

1. Rinse the plant tissues until they are free of soil.
2. Weigh the tissues to be processed and place them in the blender. Use not more than 50 g of tissue (fresh-weight basis) with 200 ml of water in 1.9-L (2-qt) blender.
3. Homogenize the mixture for 15 sec.
4. Decant the suspension from the blender, including the rinse water, over a 325-mesh sieve.
5. Using a wash bottle containing antibiotic solution (see shaker method), wash the debris from the sieve into a beaker.
6. Pour the liquid that passed through the sieve over the sieve again.
7. Combine the material collected on the sieve in the second passage with that from the first passage.
8. Gently decant the suspension of material collected on the sieve over the filter of the Baermann apparatus.
9. Fill the Baermann apparatus with enough antibiotic solution to barely submerge the debris on the filter.
10. Replenish the incubation solution with water as needed.
11. Collect the nematodes after incubation at 21–24°C for 2–3 days.

In addition to the combined procedure just described, blending followed by wet sieving is useful for certain nematodes such as *Radopholus similis* on banana.

EXTRACTION OF NEMATODE EGGS

Elutriation-Dissolution

Variation of egg numbers in the field sometimes causes problems with this assay method of elutriation, dissolution of gelatinous matrices of egg masses, and staining (5). The method is useful, however, for midseason to late-season assays of *Meloidogyne* spp. and other nematodes that form egg masses and/or cysts.

Equipment: Sample splitter or semiautomatic elutriator, 150- and 600-ml beakers, stirrers, 15-cm household sieve, 40- and 500-mesh sieves, exhaust hood, 5-ml dipper, compressed air (for cleaning sample splitter and use with elutriator).

Chemicals: Sodium hypochlorite (NaOCl), antifoam spray, acid fuchsin, lactic acid.

Procedure:

1. Mix the soil.
2. With water flow adjusted to 350 ml/sec, or 60–80 ml/sec if air-water mixture is used, place a 500-cm^3 aliquant of soil in the elutriator. Turn the water on for 2–3 min, trapping root fragments on a 40-mesh sieve.
3. With a spray nozzle, wash the residue off the sieve into a 600-ml beaker and add water to 200 ml.
4. Add 20 ml of 5.25% NaOCl and spray with an antifoam agent.
5. Stir the mixture under an exhaust hood for 10 min.
6. Using a household sieve to retain debris, take a 5-ml sample with a dipper and rinse into a 150-ml beaker.
7. Pour this subsample onto a 500-mesh sieve and wash the eggs from the sieve into a clean 150-ml beaker (should have 20–25 ml of suspension).
8. Add two drops of 0.35% acid fuchsin in 25% lactic acid and boil for 1 min under the exhaust hood (a microwave oven is useful).
9. Allow the mixture to cool before counting.

Centrifugation

Nematode eggs may be extracted by centrifugation (12,22).

BIOASSAYS OF NEMATODE POPULATIONS

With low natural populations of nematodes such as *Meloidogyne, Heterodera,* and *Ditylenchus* spp., bioassays (19,22,26) are the most reliable procedures. The following is a typical bioassay for *Meloidogyne.*

Equipment: Fumigated sandy loam soil, 10-cm clay pots, 15-cm plastic pots, pot labels, 3-week-old Rutgers or other susceptible tomato seedlings, nutrient solution, greenhouse space. Other recommended tomato cultivars are Person A1, Heinz 1350, Marglobe, Bonny Best, and Manapal.

Procedure:

1. Fill the bottom 2 cm of 10-cm clay pots with sterile soil.
2. Add 250 cm^3 of test soil to the clay pots. The field soil used for a bioassay should represent an entire plot or a portion of a given field. Large fields should be marked off into units of about 1 ha with two composite (20-core) samples used for bioassays from each unit (19). This sampling-bioassay scheme gives a ratio of standard error to mean of about 25%. More bioassays are needed if greater precision is desired (19).
3. Transplant a tomato seedling to each pot, and fill the remainder of each pot with sterile soil (place each 10-cm pot in a 15-cm plastic pot to minimize contamination).
4. Grow plants for 5–6 weeks at 24–28°C, providing nutrients and water as needed. Do not overwater.
5. Harvest the plants by washing the roots out carefully. Rate the nematode development by using the gall index (Table 4) or by determining the numbers of

eggs (see NaOCl method).

The third root-knot index (1–6) in Table 4 (nomograph) is recommended for evaluating the effects of nematicides on *Meloidogyne* spp. treated in the field or greenhouse. Figure 5 illustrates examples of each class. The other schemes in the nomograph (Table 4) are also acceptable.

Rating galling caused by different *Meloidogyne* spp. may be confusing, because the number and size of galls incited by a given number of juveniles vary with species and host plant. Concentrating primarily on the proportion of roots galled rather than the size of galls can minimize this problem.

When the cause of galls is uncertain, roots should be stained with 0.05% acid fuchsin and cleared in glycerin or lactophenol (6). The contents of galls (numbers of juveniles, eggs, and adults) can then be determined.

Gall indexes for *Meloidogyne* spp. should be limited to galling. To rate necrosis associated with root knot, a separate rating system such as the one Powell et al (21) developed is suitable. In their classification system, 0 = no necrosis, 1 = less than 10% of the root system necrotic, 2 = 11–25% necrotic, 3 = 25–50% necrotic, 4 = 51–75% necrotic, and 5 = 76–100% necrotic. Based on all root systems per treatment, the disease or necrosis index is computed as follows:

$$\mathrm{DI} = \frac{(n_1 \times 1) + (n_2 \times 2) + \ldots + (n_5 \times 5) \times 100}{5N},$$

where DI = disease index; n_i = number of plants in class i, $i = 1, 2, \ldots, 5$; and N = number of plants in treatment ($N = n_1 + n_2 + n_3 + n_4 + n_5$). A similar lesion or necrosis index is also useful for rating banana roots for infection by *Radopholus similis*.

Numbers of cysts may be counted on bioassay plants for *Heterodera* spp. (cereal cyst nematode, *H. avenae*, on wheat [26] or soybean cyst nematode, *H. glycines*, on soybean).

REPORTING RESULTS

The following results should be determined for each replicate and averaged for each treatment:

1. Mean numbers of nematodes per 100 cm^3 or greater volume of soil (use of a mechanical stage on the microscope to control counting dish increases efficiency in making nematode counts).

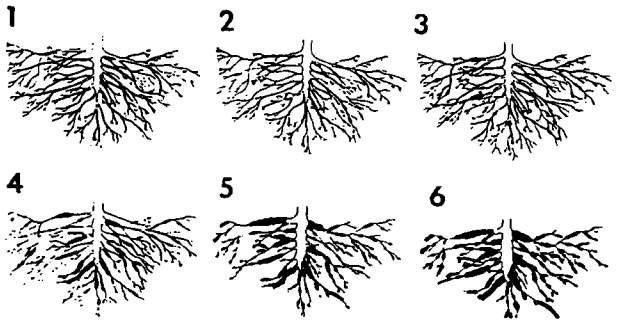

Fig. 5. Scheme for rating field and greenhouse infestation and bioassay evaluation of *Meloidogyne* spp. See Table 4 (galling index system 1–6) for descriptions of each root-knot nematode gall index class.

2. Numbers of nematodes per gram of root or other plant part (fresh or dry weight) when endoparasites are extracted from same plots. Total nematodes per plant should be included for greenhouse experiments.

3. For *Heterodera* spp., numbers of juveniles and cysts as well as numbers of eggs per cyst or per gram of soil.

4. For *Meloidogyne* spp., numbers of eggs and juveniles per unit volume of soil or weight of roots (fresh or dry weight); also record root-knot indexes based on the index given under bioassay procedure. Table 3 summarizes the data required for various types of nematodes and host plants.

5. Plant response.

Precision in determining nematode population responses to nematicides depends on how the samples are collected, handled, stored, extracted, and counted. With proper execution of each of these activities, variation usually ranges from 10 to 30%. Oostenbrink found that the usual coefficient of variation for this error is about 25%. This error varies, however, with nematode species and population densities. In estimating egg numbers for *Meloidogyne* spp., this coefficient of variation is considerably greater. Thus, proper statistical analyses of all data must be used to estimate the variance for a given experiment, including coefficient of variation and standard error. Because some zero readings and considerable variation are normally encountered, the $\log_{10}(P + 1)$ or another transformation should be used for nematode population data. Standardization of reporting nematode population data is needed to facilitate communication regarding nematicide efficacy, especially because most assay procedures vary in efficiency (22). The extraction technique and rates of recovery for each target species for the soils involved should be noted in all nematicide evaluations.

GLOSSARY OF DESCRIPTIVE TERMS

Bioassay. Use of appropriate hosts to determine relative population levels (has been used primarily for endoparasites such as root-knot and cyst nematodes, but can be used for any nematode under suitable propagation conditions; usually done in greenhouse).

Detection level. Minimum population density of a nematode species that can be identified routinely from a soil or plant tissue sample (varies with each sampling and extraction technique).

Economic threshold density. Minimum number of nematodes per unit volume of soil (or weight of plant tissue) required to cause an economically significant loss in crop production.

Fumigant nematicide. Gas, volatile liquid, or solid that diffuses through the soil pore spaces as a vapor and is water-soluble.

Grid or systematic sampling (stratified). Collection of specified number of borings over entire plot area (one for each square meter or other area measure) for pretreatment sampling. If crops are established, collection of borings from root zone (in the rows for row crops).

Horizontal head. For centrifuge, head is so structured that individual tubes (swinging bucket) are in horizontal position when centrifuge is operating.

Molar solution. 1 g mol wt in sufficient water to make 1 L of solution.

Multipurpose chemicals. Of three basic types:

Fumigant. Volatile chemical that disperses throughout the soil and controls nematodes, fungi, bacteria, insects, weeds, or a combination of these.

Nonfumigant nematicide (contact nematicide). Nonvolatile chemical that does not disperse readily except through soil water but controls nematodes (and in some cases, soil insects) that come in contact with it.

Systemic nematicide. Chemical that is absorbed by a plant and translocated throughout the plant and controls nematodes.

Nonfumigant nematicide. Nematicide that has little or no volatility and that must be mixed thoroughly in soil as a granular formulation or applied in water (water-soluble formulation).

Population density. Number of nematodes per unit volume of soil or weight of plant tissue.

ppm. Parts per million; 1 ppm = 1 mg/L (1 lb/120,000 gal) of water. With water, 1 ppm is equivalent to 1 μg/ml (w/v) or μg/g (w/w). We recommend using μg/ml or μg/g instead of ppm. The solvent should be specified when solutions other than water are used.

Random sampling. Points for collecting borings determined by random chance (not usually suitable for nematodes such as *Meloidogyne* spp., which occur in aggregates).

Relative *g* force (relative centrifugal force). For centrifuge, force (minimum, average, maximum) times that of gravity: relative g force = $0.00001118 \times r \times \text{rpm}^2$, where r = radius in centimeters.

Sampling tube. Metal (all or part) tool for collecting soil cores of constant diameter and volume. Major types are the Oakfield soil sampling tube (2.0-cm ID, open side, 38 cm long; good for routine sampling to a depth of 20 cm); the Viemeyer soil sampling tube (2.5-cm ID, 120 cm long, with reinforced head; tube can be driven into soil to a depth of 90 cm); the can or bucket auger (steel auger with bucket 5–7.5 cm in diameter by 15 cm high, fitted with cutting edges at the base; useful for obtaining large soil samples and roots to depths of 90–100 cm); and the cone-shaped sampling tube (useful for routine sampling of loose, sandy soils) (22).

Sieve mesh number. Number of wires (filaments running in each direction) per inch. We recommend that the size of openings be given in micrometers and that mesh number be given in parentheses.

Soil sterilant. A chemical (e.g., methyl bromide) that kills all soil organisms.

Tolerance limit. Minimum number of nematodes that inhibit plant growth (number per unit volume of soil or weight of plant tissue). May not have economic significance.

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NCSU IR-4 Center Form 8.4-A: Forced-air drying of PRE samples.

Trial (Chemical/Crop/Field ID):_____

Sample ID:	**Sample ID:**	**Sample ID:**	**Sample ID:**
Oven ID:	**Oven ID:**	**Oven ID:**	**Oven ID:**
Temperature: C F	**Temperature:** C F	**Temperature:** C F	**Temperature:** C F
Date and time IN:	**Date and time IN:**	**Date and time IN:**	**Date and time IN:**
Initials:	**Initial:**	**Initials:**	**Initials:**
Wet weight: g lb	**Wet weight:** g lb	**Wet weight:** g lb	**Wet weight:** g lb
Date and time OUT:	**Date and time OUT:**	**Date and time OUT:**	**Date and time OUT:**
Dry weight: g lb	**Dry weight:** g lb	**Dry weight:** g lb	**Dry weight:** g lb
% Moisture:	**% Moisture:**	**% Moisture:**	**% Moisture:**
% Dry matter:	**% Dry matter:**	**% Dry matter:**	**% Dry matter:**
Initials:	**Initials:**	**Initials:**	**Initials:**

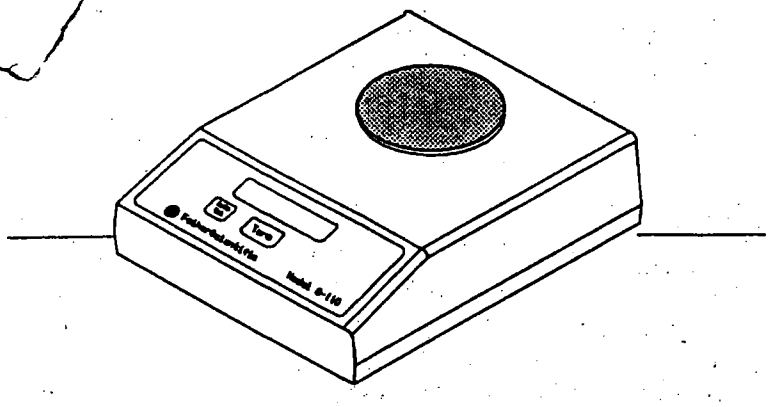
Sample ID:	**Sample ID:**	**Sample ID:**	**Sample ID:**
Oven ID:	**Oven ID:**	**Oven ID:**	**Oven ID:**
Temperature: C F	**Temperature:** C F	**Temperature:** C F	**Temperature:** C F
Date and time IN:	**Date and time IN:**	**Date and time IN:**	**Date and time IN:**
Initials:	**Initial:**	**Initials:**	**Initials:**
Wet weight: g lb	**Wet weight:** g lb	**Wet weight:** g lb	**Wet weight:** g lb
Date and time OUT:	**Date and time OUT:**	**Date and time OUT:**	**Date and time OUT:**
Dry weight: g lb	**Dry weight:** g lb	**Dry weight:** g lb	**Dry weight:** g lb
% Moisture:	**% Moisture:**	**% Moisture:**	**% Moisture:**
% Dry matter:	**% Dry matter:**	**% Dry matter:**	**% Dry matter:**
Initials:	**Initials:**	**Initials:**	**Initials:**

NCSU IR-4 Center Form 8.4-B: Forced-air drying of RAC samples.

Trial (Chemical/Crop/Field ID):_____

Sample ID:	**Sample ID:**	**Sample ID:**	**Sample ID:**
Oven ID:	**Oven ID:**	**Oven ID:**	**Oven ID:**
Temperature: C F	**Temperature:** C F	**Temperature:** C F	**Temperature:** C F
Date and time IN:	**Date and time IN:**	**Date and time IN:**	**Date and time IN:**
Initials:	**Initials:**	**Initials:**	**Initials:**
Wet weight: g lb	**Wet weight:** g lb	**Wet weight:** g lb	**Wet weight:** g lb
Target dry weight range: **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb
Date and time OUT:	**Date and time OUT:**	**Date and time OUT:**	**Date and time OUT:**
Dry weight: g lb	**Dry weight:** g lb	**Dry weight:** g lb	**Dry weight:** g lb
% Moisture:	**% Moisture:**	**% Moisture:**	**% Moisture:**
% Dry weight:	**% Dry weight:**	**% Dry weight:**	**% Dry weight:**
Within target range? y n	**Within target range?** y n	**Within target range?** y n	**Within target range?** y n
Initials:	**Initials:**	**Initials:**	**Initials:**

Sample ID:	**Sample ID:**	**Sample ID:**	**Sample ID:**
Oven ID:	**Oven ID:**	**Oven ID:**	**Oven ID:**
Temperature: C F	**Temperature:** C F	**Temperature:** C F	**Temperature:** C F
Date and time IN:	**Date and time IN:**	**Date and time IN:**	**Date and time IN:**
Initials:	**Initials:**	**Initials:**	**Initials:**
Wet weight: g lb	**Wet weight:** g lb	**Wet weight:** g lb	**Wet weight:** g lb
Target dry weight range: **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb	**Target dry weight range:** **Lower:** g lb **Upper:** g lb
Date and time OUT:	**Date and time OUT:**	**Date and time OUT:**	**Date and time OUT:**
Dry weight: g lb	**Dry weight:** g lb	**Dry weight:** g lb	**Dry weight:** g lb
% Moisture:	**% Moisture:**	**% Moisture:**	**% Moisture:**
% Dry weight:	**% Dry weight:**	**% Dry weight:**	**% Dry weight:**
Within target range? y n	**Within target range?** y n	**Within target range?** y n	**Within target range?** y n
Initials:	**Initials:**	**Initials:**	**Initials:**



SET-UP (Installation)

- Remove balance and all accessories from carton. There are no internal packing or tie downs inside the balance.
- Set voltage select switch to proper voltage.
- Select a suitable work surface.
- Place the weighing pan in the receptacle on the top of the balance.
- Insert power cord into the terminal at the rear of the balance, *Figure #1.*
  Firmly push the plug into the terminal for proper contact.
  Plug power cord into a 115 VAC or 230 VAC receptacle.

NOTE: Be sure voltage switch is set for proper voltage prior to plugging in.

- Turn the balance on with the ON/OFF switch located on the back panel, *Figure #1.*
  When power is first applied, the microcomputer performs a self-diagnostic test and then proceeds to a lamp test with all 8's appearing in the display window until stability has been obtained.
  Stability will be indicated by a lower case *g* indicating the gram mode when first turned on.

Allow a thirty minute warm-up period and make no adjustments to the balance during this time. Anytime the balance is turned OFF, the thirty minute warm-up is required. We suggest that the balance be left on continually to eliminate the warm-up period.

Fisher *SERIES S* Electronic Balance

LIMITED WARRANTY

Denver Instument Co. warrants electronic top loac ances against defects in material an workmansh period of one year from the date of original purcha: VIDED THE BALANCE IS MAINTAINED AND U ACCORDANCE WITH THE OPERATIONS INSTRU SUPPLIED WITH THE UNIT. A properly comple returned Warranty Card can expedite any service r

THE WARRANTY STATED HEREIN IS IN LIEU OTHER EXPRESS OR IMPLIED WARRANTIES THERMORE, DENVER INSTRUMENT CO. WILI EVENT BE LIABLE NOR OBLIGATED FOR ANY (QUENTIAL, INCIDENTAL, OR SPECIAL DAMAC

SHIPPING INSTRUCTIONS

1. Record the serial number of the unit.
2. Write a detailed description of the unit malfunc the packing slip.
3. Remove the weighing pan from the balance.
4. Place the balance in the original shipping carto Secure the unit with the original packing mater
5. Place the weighing pan on top of the inserts an with tape.
6. If the original packing is not available, select a c approximately the same size as the original, e adequate room for sufficient padding material (epifoam, etc.).
7. Enclose the packing slip.
8. Close the carton and secure with tape.

Denver Instrument Company
6542 Fig Street • Arvada, Colorado 80004
303/431-7255 • 1-800-321-1135 • FAX 303-423-4831

Fisher *SERIES S*
Electronic Balances -- Specifications

MODEL	CAPACITY	RESOLUTION	WEIGHING PAN DIAMETER
S-110	110g	0.001g	3 1/4"
S-400	400g	0.01g	4"
S-300D	30/300g	0.001/0.01g	3 1/4"

COMMON SPECIFICATIONS

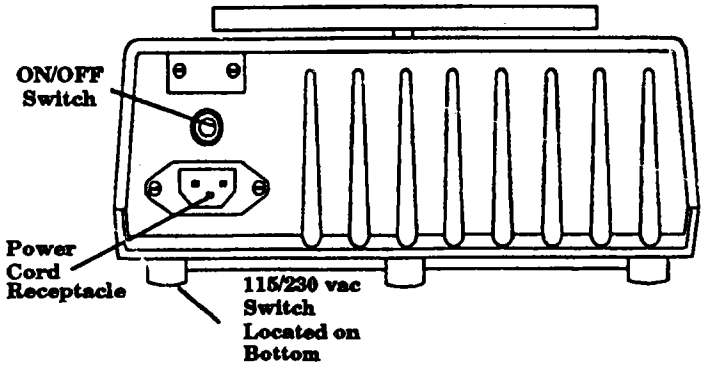
Response Time..... 1-3 seconds..... constant ON
or allow 30 min. warm-up period when turned ON
Electrical Requirements..... 115/230vac, 50/60 Hz
with 3 prong plug
Auto Cal..... all models
Overall Dimensions..... 3 3/4" x 6 1/4 " x 10 1/2"

BALANCE LOCATION (work area)

Select an environment suitable for precision weighing.

- Work area should be relatively free from drafts and vibrations. (Breeze shields are available for all models optionally.)
- Work surface should be level and rigid.
- Line voltage to the balance should be reasonably constant and free from fluctuations. It is **not** advisable to use an outlet that is shared with fluorescent fixtures or other electrical equipment that draws voltage in an inconsistent manner.
- Do **not** locate near magnetic materials or equipment/instruments which incorporate magnets in their design.
- Excessive room temperatures above 85° F/29° C could affect balance operation and accuracy.

Figure #1



TARING/ZEROING

To Tare (zero) the balance. simply press the Tar
audible Beep and all *0*'s on the display indicate the
weight has been subtracted. A quick depression
may **not** tare the balance.

The *S SERIES* have tare capabilities up-to-their t
ity. For obtaining actual weight of more than on

Place sample container on the weighing pan.
Tare balance to *0*.
Place sample to be weighed in the container an
the results. The LED shows only the weight o
sample placed in the container.

Continuous taring may be done by weighing
samples until the total capacity of the balance
reached. When the capacity is exceeded, the LED
EEEEEEEE.

AUTO-CALIBRATION

The Auto Cal allows the user to permanently chang
factor adjustment of the balance without making
ometer adjustment or using other equipment. The
necessary is a standard weight of a correct value
Cal, permissible weights chart) for each model o
The procedure is:

1. After a suitable warm-up, press Tare key and zero unit.
2. Press Auto Cal key. Display shows *CAL-F*.
3. Place a standard weight on the pan. (See Permissible Weigh
4. The display shows *CAL* and a single digit numeric value corre
the standard weight. When calibration is complete, the stan
value is displayed. (For example, if a 100 gram weight is
shown in the display window.)
5. Remove the standard weight. Tare unit. Place standard
weight back on pan to check and ensure calibration has been
completed.
6. Remove sample weight and proceed with sample weighings
reason calibration is not completed, the balance continues
before calibration. See REASONS FOR INCOMPLETE
SECTION.
7. Values used in Auto Cal are stored in memory and will not
the unit is turned off or unplugged.

Auto- Calibrate, Permissible Weights

Model	Capacity grams	AutoCal Weig grams
S-110	110	20,30,50,100
S-300D	30/300	30,100,200,300
S-400	400	100,200,300,40

Revision # 1-25-18

IR4 Storage Unit Designations

(R = Refrigerator F = Freezer A = Ambient)

Unit #	Bldg/Room	Type	Purpose	Cor
1	493	F - walk in	Sample Receiving	D/C on 2-17-17 = repl
41	685/20	R - upright	Extra Storage	D/C on 3-8-06 = replac
5	685/20	F - upright		
9	685/20	R = upright	Sample Extracts	
10	493	F = walk in	Long Term Samples & Excess Control	
11	685/20	R = upright	Neat Stds, Std. Sols, Chem. Storage	D/C on 3-07 = replace
12	816/2	F = walk in	Active Projects Samples	
14	685/20	F = upright	Std. Solutions	
15	685/20	F = upright	Neat Stds. & Archived Stds.	
16	685/20	A = cabinet	Neat Stds.	
17	685/20	F = upright	Extra Storage	Replaces # 5
18	685/20	R = upright	Neat Stds., Std. Sols, Chem. Storage	Replaces # 11
19	685/20	F = upright	Temporary Sample Storage	
20	493	F = walk in	Sample Receiving	Replaces # 1