

SOP Log Sheet

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Juliet Thompson <jthomp24@ncsu.edu>

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1 message

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Tue, Apr 22, 2025 at 9:23 AM

To: jmazlo@ncsu.edu, jthomp24@ncsu.edu

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Name: Nicole Soldan

Email: schroe65@msu.edu

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**MICHIGAN STATE
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TO: Scott Chapman
U. of Wisconsin Dept. of Entomology
1630 Linden Drive
Madison, WI 53706

FROM: Nicole Soldan, IR-4 NC Regional Field Coordinator

SUBJECT: STANDARD OPERATING PROCEDURE APPROVAL
Version 7.5

DATE: April 22, 2025 *Nicole Soldan*

Per 40CRF160 Good Laboratory Practice Standards (GLP), this is to notify you that your Standard Operating Procedure (SOP) in use is approved. Please retain this document with your SOP to fulfill GLP requirements.



**IR-4 North
Central Region
Field
Coordinator's
Office**

**Department of
Entomology**
1066 Bogue St.
Rm A448
East Lansing, MI 48824

SOP	VERSION	REVIEW DATE	SOP	VERSION	REVIEW DATE
#1	7.5	4-15-25	20	7.5	4-15-25
2	7.5	4-15-25	21	7.5	4-15-25
3	7.5	4-15-25	22	7.5	4-15-25
4	7.5	4-15-25	23	7.5	4-15-25
5	7.5	4-15-25	24	7.5	4-15-25
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9	7.5	4-15-25	28	7.5	4-15-25
10	7.5	4-15-25	29	7.5	4-15-25
11	7.5	4-15-25	30	7.5	4-15-25
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19	7.5	4-15-25			

STANDARD OPERATING PROCEDURE



UNIVERSITY OF WISCONSIN
IR-4 RESEARCH CENTER

STANDARD OPERATING PROCEDURE
VERSION 7.5 – April 15, 2025

UNIVERSITY OF WISCONSIN
IR-4 RESEARCH CENTER

These SOP's of the University of Wisconsin IR-4 Research Center replace the previous Version 7.4 SOP's dated February 5, 2024. They will be used for GLP trials conducted under the direction of Field Research Directors D.J. Heider and S.A. Chapman. They are submitted as a complete set (Version 7.5) and become effective when approved by the IR-4 Regional Coordinator.

SOP's Reviewed by:



Daniel J. Heider
Field Research Director

4/15/25
Date



Scott A. Chapman
Field Research Director

4/15/25
Date

University of Wisconsin IR-4 Research Center
STANDARD OPERATING PROCEDURE INDEX

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The above SOP's approved by:


IR-4 Regional Field Coordinator

4-22-25
Effective Date

Upon signature of the Regional Field Coordinator (per 40CFR Part 160 Good Laboratory Practice Standards - GLP) this standard operating procedure (SOP) is approved for use. This index is retained with the SOP to fulfill GLP requirements.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #1. Development and use of Standard Operating Procedures in the evaluation of pesticides.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To provide guidance in the development and use of Standard Operating Procedures in field research.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Studies, which are conducted in the support of the registration of pesticides, will have SOP's for all phases of the research. Studies, which need to be conducted under GLP Standards, will have a Quality Assurance Unit (QAU) that will periodically review the research to ensure that studies are being conducted according to protocol and all applicable SOP's. All individuals involved in IR-4 projects conducted by the Field Research Directors listed on page 2 of this SOP will follow these SOP's.

2. Each SOP will be reviewed annually by the Field Research Directors. Necessary changes will be made, and the entire set will be submitted to the IR-4 Regional Field Coordinator for approval. SOP's that are no longer applicable will be retired and not included in the new revision sent to the IR-4 Regional Field Coordinator, although they will still be listed in the index and the associated unique SOP # will not be reused.

3. Any deviations from protocol or SOP's must be documented in writing, signed and the Study Director notified as soon as possible.

4. The following is the format to be used for each Standard Operating Procedure.

SOP #: (SOP number in numerical order [1 to n]). Title: (SOP title).

Written By: (Name of person developing the SOP).

Purpose: (Brief description of the purpose of the SOP).

Scope: (Determines where and when the SOP is applicable).

Procedures: (Description of the operating procedures in numerical order from beginning to end so that a person with some knowledge of the situation can carry out the procedures without any additional information from other sources).

Version #: (serially by year following the format: 2021 = version 7.1, 2022 = version 7.2 and so on). The version # and date of annual review/submission will be listed at the lower portion of the page for each SOP.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #2. Responsibilities of the Field Research Director.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To identify the responsibilities of the Field Research Director.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: The Field Research Director has the responsibility for the following:

1. Review protocol and assure that the study is carried out according to that protocol.
2. Review and sign field data notebook at the completion of the field phase of the study.
3. Assure that personnel (Appendix A), resources, facilities (Appendix B), equipment, materials and methods are available as required for the conduct of the project.
4. Make sure that all personnel conducting the study have had sufficient training, understand the study protocol and SOP's, and have adequate resources to complete the study.
5. Respond to and act upon findings reported by the Quality Assurance Unit (QA).
6. All raw data including true copies, summaries and other items connected with the study will be forwarded to the Regional Field Coordinator or IR-4 HQ upon completion of the study as part of the field data notebook. Raw data not required as part of the field data notebook will be sent directly to IR-4 Headquarters for archiving.
7. Provide a current curriculum vitae containing education, training and experience records of trial personnel.
8. Maintain logs on critical equipment and test instruments used in carrying out the study.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #3. Site selection and plot design for field studies.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure test plots are of sufficient size and uniformity to obtain required data or residue samples.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Site selection will be made in accordance with accepted cultural practices for the commodity.
2. Site will be large enough to accommodate the required number of replicates, buffer zones and treatments in accordance with an approved protocol and for the commodity to be grown under commercial conditions yielding samples of sufficient size for analysis where required.
3. Site will be located with sufficient isolation to minimize the possibility of contamination from external sources. The minimum buffer between treated and untreated plots will reflect those listed in the protocol or will be at least 15' if no direction is provided. When the test site is located within a commercial field, buffer areas may contain a crop. These areas may be treated with maintenance chemicals if they do not compromise the integrity of the study as specified in SOP #4, part 7.
4. If the commodity is not to be newly established, select a site that appears to have uniform stand for production.
5. Acceptable cultural practices for the commodity will be followed.
6. The experimental design and number of replications as specified in the protocol will be used and documented.
7. A plot map will be prepared showing the location of each plot, the North azimuth, slope, and if possible, distances from permanent reference points to plot corners. Differential GPS, accurate to within one meter, may instead be used to mark plot or study corners. If differential GPS is used, the SOP for its operation, maintenance and standardization will be followed.
8. Plots in the field will be labeled with stakes or flags with at least one marker indicating the study number and treatment. The plot map will be labeled with sufficient information to identify the treatment assigned to each plot.
9. Each plot will be laid out using measuring devices capable of accurately locating the plots on the site.
10. Test site history (crops, pesticide and fertilizer use) will be obtained for a minimum of one year for the test site.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #4. Commodity establishment and maintenance.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that commodities are grown under good agricultural practices and provide a uniform crop for study.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Utilize up-to-date information for production of the study commodity. If unfamiliar with the study crop, consult with agricultural specialists familiar with the production practices required to produce the commodity.
2. Prepare the test site as specified for the commodity.
3. If possible, obtain representative samples of soil from the test site and have them analyzed to verify that the soil meets commodity requirements and that soil fertility was not a limiting factor for normal growth and development. The analysis should include pH, soil characterization, cation exchange capacity and any other parameters required by the protocol. A soil sample for a given site will be considered valid for 3 years unless the protocol provides differing guidance. Soil sampling is not required for greenhouse trials with purchased potting media.
4. Determine a suitable crop variety and type to use for the study within the guidelines specified by the study protocol. If the variety is not specified, use any available variety suitable to local conditions and acceptable to commercial producers. Record: variety, seed source and lot # of seed if available in the field data notebook.
5. Follow protocol if specified or utilize common production practices for: plot size, row spacing, in – row seed spacing and seeding depth. Record required information in the field data notebook.
6. Irrigate or perform other agricultural practices as necessary to establish and maintain the crop through harvest. Document this information in the field data notebook.
7. Pesticides may be applied to the commodity to prevent losses due to pests and will be applied according to the relevant SOP's in this document. Maintenance applications will be recorded in the field data notebook. No pesticide will be applied that would interfere with the chemical analysis of the pesticide under study. The study director will be contacted if there is concern of a possible interference from maintenance pesticide applications.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #5. Standardization of instruments used to monitor environmental conditions.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that all weather instruments used in field research studies are reasonably accurate and in good working order.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be performed at the beginning of each spray season. Fill out a standardization form for each instrument tested with all pertinent information and when complete, place the form in the field data notebook.

A. Standardization of electronic thermometer with probe.

1. Visually inspect thermometer for signs of obvious wear or breakage. Replace batteries as needed.
2. Turn on the thermometer and compare reading of the ambient air temperature with that obtained with a general laboratory thermometer kept as a standard. Record readings on the standardization form. Move thermometers to a second temperature point like a refrigerator or stabilized ice bath and again record the readings on the standardization form.
4. Electronic probe thermometer readings obtained at any temperature point should not vary by more than $\pm 3^{\circ}\text{F}$ from the reference thermometer.
5. If the thermometer gives inconsistent results or results not accurate to within desired tolerances, it will be replaced.

B. Standardization of the electronic recording datalogger for test substance storage.

1. Visually inspect datalogger for signs of obvious wear or breakage. Monitor battery levels during the season and replace as needed.
2. The datalogger will be programmed to record the ambient temperature every 30 minutes.
3. Check standardization by placing the laboratory thermometer and the datalogger in close proximity and manually record a total of 3 readings over a range of temperatures on the standardization forms. After collecting the data, download the electronic recording datalogger and print the temperature file. Transcribe the recorded temperatures on the standardization form.
4. Compare the readings collected from the reference thermometer and those collected electronically. If electronic readings vary by more than $\pm 3^{\circ}\text{F}$ from the reference thermometer, replace or calibrate the electronic recording datalogger.
5. Include the electronic data printout for the standardization period in the field data notebook on the standardization form or print separately and include.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #5. Standardization of instruments used to monitor environmental conditions (*continued*).

C. Standardization of the electronic anemometer.

1. Visually inspect the anemometer for signs of wear or breakage. Replace batteries as needed.
2. Turn instrument on to the mph setting.
3. Face the wind (if wind is calm, a fan may be used) and hold the anemometer side by side with an identical reference wind gauge kept solely for the standardization procedure.
4. Record readings from both anemometers on the standardization form.
5. If readings differ by more than 2.0 mph, replace the electronic anemometer with a new one.

D. Standardization of the electronic hygrometer.

1. Visually inspect the hygrometer for signs of wear or breakage. Replace batteries as needed.
2. Turn on the hygrometer.
3. Record % relative humidity reading obtained from the hygrometer being tested.
4. Obtain % relative humidity reading from reference electronic hygrometer used only for the standardization procedure (alternatively a sling psychrometer may be used to obtain reference humidity readings if desired) and record reading on the standardization forms.
5. If readings from the test hygrometer vary by more than ± 5 humidity units (%) from the reference hygrometer, check both instruments for accuracy against humidity readings obtained from a sling psychrometer. Replace or recalibrate any instrument where readings vary by more than ± 5 humidity units (%) from those obtained from the sling psychrometer.

E. Standardization of an electronic thermometer without probe.

1. Visually inspect the electronic thermometer for signs of wear or breakage. Replace batteries as needed.
2. Turn on the thermometer and compare reading of the ambient air temperature to temperature obtained with a general laboratory thermometer kept as a standard. Record both readings on the standardization form.
3. Repeat the process described in #2 but vary temperature conditions for subsequent tests. Record these readings on the standardization form.
4. If electronic thermometer readings vary by more than $\pm 3^{\circ}\text{F}$ from the reference thermometer, replace the electronic thermometer.

F. Environmental weather monitoring.

1. Weather information for the test site will be obtained from the closest permanent recording station. These stations may be operated by experiment station personnel, NOAA, the cooperating grower, or other trusted entities. If possible, rainfall and irrigation data will be obtained from the cooperating grower at the test site.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #6. Calibration of tractor plot sprayer.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To determine the delivery rate of the plot sprayer and make adjustments as necessary to ensure an accurate application of the pesticide.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be performed at the beginning of each spray season and within one day prior to application of a pesticide to a residue plot.

1. Visually inspect hoses, pipes, fittings, regulators, gauges, nozzles, screens and tanks for wear, cleanliness and potential leaks. Repair or replace as necessary and record in the sprayer maintenance log.

2. Calibration is performed in the following manner.

a. Operate tractor over a known distance at a constant speed.

b. Record distance and time for travel on the calibration form and calculate speed (**ft/sec**).

c. Clean sprayer. Fill sprayer with clean water, pressurize tanks and operate sprayer to remove air from the delivery lines.

d. Operate sprayer for a known time, collect output from each nozzle separately, record tank pressure and ml collected in the field data notebook. Complete calculations required on the discharge calibration form of the field data notebook.

e. Replace any nozzle on the boom, which varies by more than 5% from the mean boom output.

f. For hand calculated calibrations determine speed (feet/second) and determine total output (ml/second) and use these values in the following formula (modified or alternative formula's may be used at the discretion of the field research director) to determine GPA. Record all information in the field data notebook. If using the eFDB, validated calculations are provided automatically.

$$\text{Delivery Rate (GPA)} = \frac{\text{Nozzle Output}}{1} \times \frac{\text{Speed}}{1} \times \frac{\text{Nozzle Spacing}}{1} \times \frac{\text{Acre Area}}{A} \times \frac{\text{Gallon Conversion}}{3785 \text{ ml}}$$

$\frac{\boxed{} \text{ (ml/sec)}}{1} \times \frac{1}{\boxed{} \text{ (ft/sec)}} \times \frac{1}{\boxed{} \text{ (ft)}} \times \frac{43560 \text{ ft}^2}{A} \times \frac{1 \text{ Gal}}{3785 \text{ ml}}$

g. For small changes in output, vary pressure or speed slightly to obtain desired output and repeat above procedure. For larger changes, adjust nozzle size or type, or speed of travel and recalibrate.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #7. Calibration of CO² backpack plot sprayer.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To determine the delivery rate of the plot sprayer and make adjustments as necessary to ensure an accurate application of the pesticide.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be performed at the beginning of each spray season and within one day prior to application of a pesticide to a residue plot. This procedure must be performed by the person who is going to apply the pesticide.

1. Visually inspect hoses, pipes, fittings, regulators, gauges, nozzles, screens and tanks for wear, cleanliness and potential leaks. Repair or replace as necessary and record in the sprayer maintenance log.
2. Components of a backpack CO² sprayer (booms, handle, hose, regulator and spray bottle or tank) will all be labeled with the same identifier to indicate they are part of the same sprayer. Although multiple booms (different nozzle spacing and boom widths) exist for each sprayer there is only one of each combination associated with each sprayer. Therefore the exact boom used will be indicated by the nozzle spacing and number of nozzles as indicated in the field data notebook.
3. Calibration is performed in the following manner.
 - a. Using a metronome or stopwatch to check pace, record the time it takes to walk a known distance at a constant pace on the calibration form and calculate speed (**ft/sec**).
 - b. Fill sprayer with clean water, set the pressure regulator to the desired pressure, pressurize the tank and operate sprayer to remove air from the delivery lines.
 - c. Operate sprayer for a known time, collect output from each nozzle separately, record ml collected on the calibration form, and determine output (**ml/sec**).
 - d. Replace any nozzle on the boom, which varies by more than 5% from the mean boom output.
 - e. For hand calculated calibrations determine speed (feet/second) and total output (ml/second) and use these values in the following formula (modified or alternative formula's may be used at the discretion of the field research director) to determine GPA. Record all information in the field data notebook. If using the eFDB, validated calculations are provided automatically.

$$\text{Delivery Rate (GPA)} = \frac{\text{Nozzle Output}}{1} \times \frac{\text{Speed}}{1} \times \frac{\text{Nozzle Spacing}}{1} \times \frac{\text{Acre Area}}{43560 \text{ ft}^2} \times \frac{\text{Gallon Conversion}}{3785 \text{ ml}}$$

$\frac{\boxed{} \text{ (ml/sec)}}{1} \times \frac{1}{\boxed{} \text{ (ft/sec)}} \times \frac{1}{\boxed{} \text{ (ft)}} \times \frac{1}{A} \times \frac{1 \text{ Gal}}{3785 \text{ ml}}$

- f. For small changes in output, vary pressure to obtain desired output and repeat above procedure. For larger changes, adjust nozzle size or type, or speed of travel and recalibrate.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #8. Standardization of an electronic balance.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure the accuracy of any balance used in weighing the test substance.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be conducted at the beginning of every spray season, whenever the balance has been moved, and prior to the weighing of a test substance for application.

1. Visually inspect the balance for obvious problems and cleanliness.
2. Make sure balance is placed on a solid, level, clean and dry surface.
3. Connect balance to the power supply and turn on the switch.
4. Using a traceable NIST stainless steel Class F laboratory mass set, place each of the following on the balance pan and record the weights in the log: 0.10 g, 0.20 g, 0.30 g, 0.50 g, 1 g, 2 g, 5 g, 10 g, 20 g, 50 g, 100 g, 200 g, and 500 g. This procedure should be followed at the beginning of the spray season and whenever the balance is moved. Thereafter, a balance check should be performed and documented prior to measuring test substance, by bracketing the desired test substance weight if possible. The mass set will be re-certified approximately every two years.
5. If any of the weights less than 1 g varies by more than 10% or if any of the weights 1 g and larger vary by more than 1%, the balance should be calibrated using the procedure supplied in the owner's manual and rechecked. If this fails to correct the problem, the balance should be repaired at an authorized repair facility or replaced.
6. Prior to weighing a test substance for application a balance check will be done by bracketing or checking the balance readout using traceable NIST weights that are above and below the test substance weight. For example, if the test substance weight is 2.7 g, the balance will be checked using the 2 g and 5 g weights.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #9. Calculation of amount of test substance required for rates specified in protocol.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure accurate calculation of the amount of test substance needed per plot or mix size.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be conducted for determination of test substance required using hand calculations.

1. Calculate the volume of spray mixture required to treat the test plots using the following formula (the formula may be modified or changed at the discretion of the field research director).

$$\text{Gals of spray mixture} = \frac{\text{Sprayer output (GPA)} \times \text{Plot area (ft}^2\text{)} \times \text{Overage Factor}^*}{43560 \text{ ft}^2/\text{A}}$$

*This factor allows for a determined overage (the factor will be adjusted as needed), providing the extra spray volume needed to prime the spray system and maintain accurate output throughout the length of the plot.

2. Calculate the amount of test substance formulation required in the spray mixture to provide the application rate (lbs. active ingredient/A or lbs. acid equivalent/A) specified in the protocol using the following formulas (formulas may be modified or changed at the discretion of the field research director).

- a. For liquid formulations measured volumetrically.

$$\text{ml of formulated test substance} = \frac{\text{Protocol rate (lb Ai/A)} \times \text{gals of spray mix} \times 3785 \text{ ml/gal}}{\text{sprayer output (GPA)} \times \text{lb Ai/gal in formulated t.s.}}$$

- b. For dry formulations measured by weight.

$$\text{grams of formulated test substance} = \frac{\text{Protocol rate (lb. Ai/A)} \times \text{gals of spray mix} \times 454 \text{ grams/lb.}}{\text{sprayer output (GPA)} \times \% \text{ Ai in formulated t.s. (as decimal)}}$$

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #10. Measuring and transporting a pesticide formulation.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure an accurate measurement of test substance for applications of pesticides in field research. To ensure the safe transport of the test substance from storage to application.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: Check label on test substance container to make sure it corresponds to the test substance called for in the protocol and has the correct name, formulation, lot or batch number and expiration date. Record all test substance removals in the appropriate field data notebook pesticide use log.

A. Measuring Liquid Pesticide Formulations

1. Obtain a clean graduated cylinder, graduated syringe or pipette to measure the desired volume of test substance needed for the mix.
2. Select and wear appropriate safety equipment while handling the pesticide concentrate.
3. Measure the correct amount of liquid concentrate (as previously determined in SOP #9) into the cylinder, syringe or pipette. Take the reading of the liquid in the cylinder at the bottom of the meniscus.
4. Pour the liquid directly into the mixing container, transferring all of the test substance possible from the measuring device to the mixing container. Use mix water to rinse the measuring device if needed, making sure that all the concentrate has been transferred.

B. Measuring Dry Pesticide Formulations

1. The balance will be calibrated as described in the SOP #8, "Calibration of the balance". When possible, the calibration check will bracket the weight of the test substance required.
2. A plastic weighing boat or other vessel, suitable to hold the desired amount of test substance will be placed on the scale to tare it according to the manufacturer's directions.
3. Select and wear appropriate safety equipment while handling the pesticide.
4. Weigh the correct amount of concentrate (as previously determined in SOP #9) into the tared container.
5. Add the pesticide concentrate to the spray mix. If measured pesticide concentrate is to be transported, place into a suitable container and label with pesticide name, amount, and treatment number.

C. Transporting pesticides

1. When the test substance is to be applied at a plot location more than 1 mile from the IR-4 pesticide storage facility (to be accompanied with a min/max thermometer if required):
 - a. Liquid Formulations – Transport the test substance in a secure, and when possible, insulated container, taking necessary precautions to keep pesticide container in an upright position.
 - b. Dry Formulations – Transport the weighed and properly labeled amount of pesticide in a secure, and when possible, insulated container.

Standard Operating Procedure

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IR-4 Research Center

SOP #11. Pesticide application.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that pesticide treatments are safely and correctly applied according to protocol.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. All personnel involved in the mixing, application, and cleanup of pesticides are required by law to be certified by the Wisconsin Department of Agriculture, Trade and Consumer Protection.
2. The study protocol will be reviewed by study personnel to determine the proper dosage and timing of the pesticide applications.
3. Equipment used in the application of the pesticides should be inspected and calibrated in a manner acceptable for that piece of application equipment.
4. Granular materials will be applied as described in SOP #12, "Treating plots with granular pesticides".
5. Personnel mixing and applying the pesticide will wear appropriate protective clothing.
6. The amount of pesticide concentrate needed for each mix will be determined as described in SOP #9 and measured out as described in SOP #10.
7. Measure the amount of water needed to dilute the measured amount of test substance. The pH of the mix water will be determined using pH litmus paper strips. Temperature and pH of the mix water are recorded in the field data notebook.
8. Add the measured amount of test substance to the measured water and agitate the spray mix to assure an even mix of pesticide and water. If the protocol requires the use of an adjuvant (see SOP #31 regarding the storage and measurement of adjuvants) add the adjuvant to the mix at the appropriate time (recording pertinent information in the field data notebook) and continue to agitate.
9. Add mixture to the spray tank, if mixed in a separate container.
10. Make sure all settings of pressure and speed are set according to specifications from the calibration as previously performed.
11. Apply the material beginning with the treatment of lowest concentration and work up to the treatment containing the highest concentration.
12. Just before entering each plot make sure you are traveling at the correct speed and turn on the sprayer. When spray boom crosses the front edge of the plot, begin timing application with a stopwatch if pass times are required for back-calculation of rate of pesticide application. Maintain the correct constant speed through the plot.
13. When spray boom crosses the back edge of the plot, turn off stopwatch (if pass time is being recorded). Turn off the sprayer after leaving the plot.
14. Calculations should be made to minimize the amount of spray material left in the spray tank. Any leftover material will be disposed of in an acceptable manner.

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SOP #11 Pesticide application *continued*.

15. If something goes wrong during an application, such as a plugged nozzle or a broken hose, immediate action to correct the problem should be taken. The affected portion of the plot should be carefully marked with stakes or flags to indicate the area affected. This portion of the plot should not be used for obtaining samples of the commodity for residue analysis. If unaffected area is too small to obtain the samples required for analysis, then the trial should be discontinued. Appropriate individuals should be notified of the incident and details should be recorded in the field data notebook.

16. Pass time data will be collected and recorded in the field data notebook. It will be used to back-calculate the actual application. The following formulas are one example of an acceptable method of back-calculation, but may be modified at the discretion of the field research director. If using eFDB validated back-calculation is provided automatically.

Calculation of Actual Application Rate for Dry Formulation Test Substance

Boom Discharge Rate = Nozzle output _____ (ml/sec) x Number of Nozzles _____ = _____ ml/sec

Treated Area = Swath Width _____ (ft) x Plot Length _____ (ft) x No of Passes (____) = _____ ft²

Total Spray Mix Volume = Carrier Volume _____ (ml) + Adjuvant Volume _____ (ml) = _____ ml

Test Substance Concentration = _____ (%)

Actual Application Rate

Boom Discharge Rate	Total Pass Time		Test Substance Added to Mix	Test Substance Concentration as decimal)			
_____ ml/sec	_____ sec		_____ g	_____ ai			
x							
_____ ft ²		x	43560 ft ² /A x _____ ml x 454 g/lb		=		
Treated Area			Spray Mix Volume				

_____ x _____ = _____ x _____ = _____ lbs ai/A

%Application = _____ Actual Application Rate / _____ Target Application Rate x 100 = _____

%Deviation = _____

Pass Time	Boom Discharge Rate						
_____ sec	_____ ml/sec		43560 ft ²	1 gal			
x							
_____ ft ²		x	A	3785 ml	= _____ GPA		
Treated Area							

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SOP #11 Pesticide application *continued*.

Calculation of Actual Rate for Liquid Formulation Test Substance

Boom Discharge Rate = Nozzle output _____ (ml/sec) x Number of Nozzles _____ = _____ ml/sec

Treated Area = Swath Width _____ (ft) x Plot Length _____ (ft) x Number of Passes = _____ ft²

Spray Mix Volume = Carrier _____ (ml) + Test Subst _____ (ml) + Adjuvant _____ (ml) = _____ ml

Test Substance Concentration = _____ lbs ai/gal

Actual Application Rate

Boom Discharge Rate	Total Pass Time		Test Substance Added to Mix	Test Substance Concentration	
_____ ml/sec	x _____ sec		43560 ft ² /A x _____ ml	x _____ lbs ai/gal	=
_____ ft ² x 3785 ml/gal		X	_____ ml		
Treated Area			Spray Mix Volume		
_____		X	_____		= _____ lbs ai/A

%Application = _____ Actual Application Rate / _____ Target Application Rate x 100 = _____

%Deviation = _____

Pass Time	Boom Discharge Rate				
_____ sec	x _____ ml/sec		43560 ft ²	x _____ 1 gal	= _____ GPA
_____ ft ²		X	A	x _____ 3785 ml	
Treated Area					

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SOP #12. Pesticide Application of Seed Treatments

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel treating seed with a pesticide prior to planting of the seed.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Registrant treated seed:

1. If seed is to be treated by the registrant, seed of a suitable variety for the region to be grown will be procured and sent to the seed treating facility. Alternatively, the registrant or IR-4 may determine which seed is to be treated for the study.
2. Upon receipt of the treated seed, it will be stored in a monitored environment until planting. If this storage is the test substance storage, the seed will be bagged in plastic to prevent pesticide contamination. After planting unused seed may be disposed of in an acceptable manner and the containers for the treated seed will be retained until the final report has been signed by the study director.

Field Research Director treated seed:

Liquid Pesticide Seed Treatments

1. Measure out the correct amount of pesticide required for the amount of seed to be treated.
2. If pesticide requires dilution, dilute as directed by protocol or the pesticide label.
3. Apply the pesticide in a protocol acceptable manner to achieve thorough coverage of the seed, while minimizing excessive liquid application. If seed type allows, seed may be tumbled during application to facilitate thorough coverage. If seed type does not allow tumbling (i.e. potato, etc.), seed will be treated on one side, rolled over and treated on the other.
4. If seed is not to be immediately planted after treating, treated seed should be transferred to a clean container, labeled and stored appropriately until planting.

Dry Pesticide Seed Treatments

1. Measure out the correct amount of pesticide required for the amount of seed to be treated.
2. Place seed in a container of a material that will minimize pesticide adhesion to the container. Add the dry pesticide, close the container and agitate seed until the pesticide thoroughly coats the seed. Transfer the treated seed into the planter, along with any remaining dry pesticide left in the container or plant by hand.

Standard Operating Procedure

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SOP #13. Post-harvest pesticide applications

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel treating a commodity after it has been harvested from the field

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Liquid Pesticide Applications

1. Measure out the correct amount of pesticide required for the amount of commodity to be treated.
2. If pesticide requires dilution, dilute as directed by protocol
3. Apply the pesticide through a sprayer onto the harvested grain, tubers or other commodity in a manner to achieve thorough coverage, while minimizing runoff. If commodity type allows, it may be tumbled using a conveyor or other machinery during application to facilitate thorough coverage. If commodity type does not allow tumbling or is not practical, the commodity will be treated on one side, allowed to dry, then rolled over and treated on the other side.
4. After the treated commodity has been allowed to dry, the sample required by protocol will be collected into a sample residue bag.

Dry Pesticide Applications

1. Measure out the correct amount of pesticide required for the amount of commodity to be treated.
2. Place the commodity in a container of a material that will minimize pesticide adhesion to the container. Add the dry pesticide, close the container and agitate the commodity until the pesticide thoroughly coats the commodity. The sample required by protocol will then be collected into a sample residue bag.

Standard Operating Procedure

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SOP #14. Soil directed spray or soil drench pesticide applications

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel treating crop plants with a soil directed spray or soil drench application in the field

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Soil Directed Spray or Drench Applications

1. Measure out the correct amount of pesticide required to meet the protocol application rate for the number of plants or area to be treated.
2. If pesticide requires dilution, dilute with water as directed by protocol.
3. Apply the pesticide to the base of the plant (or as specified in the protocol) using a single nozzle sprayer, syringe, graduated cylinder or other measuring device to ensure that the correct amount is applied to each plant. Make the application so as to uniformly apply the test substance in all directions around the plant. If a single nozzle sprayer is used, a stopwatch should be utilized to measure the total amount of application time.
4. For applications utilizing a measuring device to apply the test substance, measure the amount of remaining pesticide (or diluted pesticide) for use in back-calculating or determining the actual amount applied per plant. For applications utilizing a single nozzle sprayer, the time of application should be recorded for use in back-calculating.

Standard Operating Procedure

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SOP #15. Granular pesticide applications.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure an accurate and safe application of granular pesticides.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: **Hand Application**

1. With the previously calibrated balance, carefully weigh out the amount of pesticide needed as specified in the protocol.
2. If plot size is large, divide plot into marked subplots to facilitate even distribution of test substance.
3. If subplots are used, weigh out the appropriate amount of test substance for each subplot.
4. The weighed-out pesticide may be extended with an inert material such as sand or calcine clay if it is of such small quantity that accurate hand application of the weighed material is impossible without doing so. The material and extender should be mixed thoroughly by hand in a clean plastic pail or plastic bag to make as uniform mix as possible. Record the material and the amount used in the field data notebook if this procedure is followed. Uniformly apply the material to the treated plot area using an appropriate container or shaker with openings of sufficient size for application of the granular test substance.
5. Apply the weighed-out test material to the correct plots (or subplots) by hand, taking care to distribute all of the material evenly over the entire plot (or subplot) without going outside the plot borders. Appropriate safety clothing should be worn when weighing, mixing and applying the test substance.

Machine Application

1. Several different types of granular applicators are available depending on the type of application specified in the protocol. Record the type of machine used.
2. Inspect the machine for mechanical function.
3. Calculate the correct amount of test substance needed to treat the desired area and calibrate the machine using the method most appropriate for the specific application prior to treatment. In general this will involve determining speed and output of the applicator in amount of test substance/measured time. If pesticide blank material is supplied by the registrant, it may be used to calibrate output of the applicator. Record calibration procedures in the field data notebook. Utilize appropriate safety equipment to calibrate and apply the test substance to the treated plot.

Standard Operating Procedure

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SOP #16. Cleanup of application equipment.

Written By: D.J. Heider and S.A. Chapman.

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

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. All personnel involved in the mixing, application, and cleanup of pesticides will be properly trained by University of Wisconsin personnel and certified by the Wisconsin Department of Agriculture, Trade and Consumer Protection.
2. Excess spray solution, pesticide rinse water and other dilute pesticide waste will be disposed of in an acceptable manner.
3. Non-disposable items will be cleaned following manufacturer's instructions or with soap and water.
4. Disposable items will be triple rinsed and disposed of appropriately.
5. Application equipment will be washed with an appropriate detergent and water, and then thoroughly rinsed prior to residue trial applications. Application equipment will be thoroughly rinsed with clean water following residue trial applications.

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SOP #17. Chemigation Applications

Written By: D.J. Heider and S.A. Chapman.

Purpose: To provide guidance to trial personnel conducting chemigation field studies

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Chemigation Applications

1. Rain gauges or water flow meters may be used to monitor irrigation applications. Rain gauges will provide direct measurement, while alternatively total irrigation may be calculated from the gallons applied over a known area.
2. If possible, operate the irrigation in advance of the application to visually verify that all sprinkler nozzles are functioning properly.
3. Measure out the correct amount of test substance required to meet the application rate specified in the protocol.
4. If the test substance requires dilution, dilute with water as directed in the protocol. Mix water pH and temperature should be recorded prior to adding the test substance.
5. Inject the test substance into the irrigation/chemigation system.
6. After all the test substance has been injected into the system, continue running the irrigation system to flush all of the test substance through the system.
7. After the irrigation has been stopped, check and record either the rain gauge collection or flow meter amount to use in determining the water (carrier) application rate.

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SOP #18. Greenhouse studies

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To ensure greenhouse facilities are sufficient to meet research needs

Scope: All greenhouse studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Each greenhouse should be large enough to contain an entire trial or portion of a trial with enough space between plots to prevent contamination. In some instances, an impervious curtain (plastic or other) may be hung between treatments within a greenhouse during application to help prevent contamination.
2. If more than one trial is conducted in a greenhouse, there should be enough space between the trials to prevent contamination or interference between trials.
3. Environmental conditions (lighting, temperature, humidity and shade) should be sufficiently uniform at the trial sites within the greenhouse to allow nearly uniform plant growth throughout the trial area. This is especially important if the trial is conducted in more than one greenhouse.
4. Fertilization events will be recorded, providing date and amount of nutrients supplied.
5. Irrigation (one of the following methods will be used):
 - a. Manual watering - Pots are watered daily by hand with a hose and nozzle to meet the needs of the developing plants. This amount changes as the season progresses and is dependent upon the experience of the individual doing the watering and light intensity into the greenhouse. The amount of water provided per plant is not measured or recorded. All irrigation events are applied as a drench to the soil at the base of the plant so that minimal foliage is contacted by the water.
 - b. Drip irrigation watering – An emitter will be placed in each pot and the water flow rate will be periodically adjusted to meet the demands of the growing plants. The amount of water provided per plant is not measured or recorded. All drip irrigation is applied to the soil so that no foliage is contacted by the water.
6. The walls, floors, and ceilings of the greenhouse should be kept in good condition. Floors, benches and aisles should be well drained and reasonably free of debris and weeds.
7. Greenhouses should be equipped to allow temperature, lighting and moisture to be maintained in a way that simulates commercial greenhouse conditions or as required by the protocol.
8. Greenhouse temperature (and if possible, humidity) will be monitored via datalogger to ensure that a proper growing environment has been maintained. Temperatures and relative humidity will be downloaded and printed at the completion of the trial and included in the field data notebook.
9. Cultural practices performed within the greenhouse will be recorded in the IR-4 field data notebook.

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SOP #19. Collection and recording of raw data

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that raw data collected and recorded is accurate and follows protocol.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Raw data will be recorded in the IR-4 field data notebook. Raw data recorded on paper will be done using indelible ink.
2. Corrections will be made by initialing, dating and providing a reason for the correction.
3. No raw data will be removed from data file.
4. Sign and date the field data notebook when prompted. If additional entries are made on subsequent dates, initial and date those entries on the dates they are made.
5. The first hard copies of electronically generated data should be signed, dated and treated as raw data.
6. If raw data is applicable to more than one study, an exact copy of the data can be substituted for the original as long as the location of the original is referenced on the copy.
7. Make sure all data required by the study protocol is collected and recorded. Carefully review the forms provided with the protocol to make sure that all the required data is being collected.

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SOP #20. Methods for collecting efficacy, phytotoxicity and yield data.

Written By: D.J. Heider and S.A. Chapman.

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

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

A. Phytotoxicity data:

1. Consult the protocol to determine the method and timing of collection for phytotoxicity ratings. List method used when recording raw data.

B. Efficacy data:

1. Consult the protocol to determine the method and timing of collection for efficacy data ratings. List method used when recording raw data.

C. Yield data:

1. Consult the protocol to determine the method and timing of harvest. If none exist, then follow commercial practices in the area for method of harvest, timing and grading of the commodity. List method used when recording raw data.

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SOP #21. Residue sample collection and storage.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that residue samples are properly collected and stored.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Consult the study protocol to establish specific dates for the collection of samples. If these dates are based on uncontrolled events (plant size, fruit maturity, etc.), then tentative dates will be established and refined as necessary.
2. Prior to sample collection, obtain a sufficient number of IR-4 supplied residue sample bags to collect all the samples.
3. Unless otherwise directed, label each sample bag using indelible ink with the following information:
 - a. Field ID Number
 - b. Crop Fraction
 - c. Test Substance
 - d. Treatment and Sample ID
 - e. Harvest Date
 - f. Sample Date
 - g. Field Research Director: Name/ Phone #
4. Harvest representative samples of the crop from each plot. Record harvest procedure used in field data notebook.
5. Consult the study protocol to determine sample size and any special handling instructions for the commodity.
6. Unless protocol directs otherwise, sample beginning with the untreated plots and working up to the highest dosage. Harvested samples will be packaged and labeled in accordance with protocol directions.
7. The following parameters will be followed in the sample collection process:
 - a. Avoid contamination of the field sample with the test substance during sampling, labeling, storage and shipping processes.
 - b. Avoid taking diseased or undersized crop parts.
 - c. Take care not to remove surface residues during handling, packing or preparation.
 - d. Use clean tools.
 - f. Document any modifications to the harvested commodity in the field data notebook.

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SOP #21. Residue sample collection and storage, *continued*.

8. Close the bag to prevent degradation of the sample under reasonable storage, handling, and transportation conditions. Excess air should be expelled from the bag.
9. When the collection is completed, the samples will be removed from the field and placed in storage as soon as practically possible.
10. Consult the study protocol for the method, temperature, and maximum length of time for storage. Frozen samples will be stored at approximately 0°F. To facilitate quick freezing, samples will be spread out on in the freezer. Untreated and treated samples will be stored on spatially apart if possible, in the freezer until packed in shipping cartons.
11. To eliminate the possibility of mixing samples from different studies, they will be packed in shipping cartons as soon as practical after the samples are frozen.
12. The freezer temperature will be recorded by means of an electronic recording datalogger to verify that the temperature is maintained within the limits as prescribed by the study protocol. Temperature files stored in the datalogger will be downloaded approximately monthly during times when residue samples are stored in the freezer (less frequently at other times of the year when no residue samples are present). It is normal for these printouts to contain temperature spikes of short duration which result when the freezer periodically goes into defrost mode. A second electronic datalogger will be utilized for backup in the event that the primary datalogger fails and will travel with the residue samples if the samples are moved to other storage in the event of freezer failure. Temperatures from the backup datalogger will only be downloaded in the event of primary datalogger failure or sample movement to temporary storage. Freezer dataloggers will be standardized annually using the following procedure:

Standardization of the electronic recording datalogger for residue sample storage.

- A. Visually inspect datalogger for signs of obvious wear or breakage. Monitor battery levels during the season and replace as needed.
 - B. The datalogger will be programmed to record the temperature every 30 minutes.
 - C. Check standardization by placing the laboratory thermometer and the datalogger in close proximity and manually record a total of 3 temperature readings on the standardization form. After collecting the data, download the electronic recording datalogger and print the temperature file. Transcribe the recorded temperatures onto the standardization form.
 - D. Compare the readings collected from the reference thermometer and those collected electronically. If electronic readings vary by more than +/- 3° F from the reference thermometer, replace or calibrate the electronic recording datalogger.
 - E. Include the electronic data printout for the standardization period in the field data notebook on the standardization form or print separately and include.
13. The following information will be recorded in the freezer contents log: 1) Trial ID No., 2) Contents, 3) date/time samples were placed in freezer, and 4) date/time samples were removed from freezer. Each entry in the log should be initialed and dated. Removal of residue samples from the freezer during packaging of samples for shipping will not be recorded if the duration outside the freezer is 3 minutes or less.

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SOP #21. Residue sample collection and storage, *continued*.

14. The primary freezer electronic recording datalogger is equipped with an alarm function capable of email and text message when temperatures exceed specified conditions. The datalogger will be set to notify the field research directors via email and text message when a temperature exceeding 7°F has been exceeded for three consecutive data points (1 hour). This large and well insulated walk-in freezer has remained below 32°F during past electrical outages for time periods of up to 24 hours. The 7°F alarm setting will provide ample time for corrective action to be taken. This alarm system will be tested annually just prior to datalogger standardization by warming the probe above 7°F for three consecutive data points to verify that a warning email and text are sent. The results of this test will be documented on the residue sample storage datalogger standardization form. If any issues are documented, corrective action will be taken, and the system will be re-tested.

15. A backup freezer will be kept in operation during the residue season for emergency storage of the samples if a failure of the primary freezer occurs (see list of backup freezers below). The residue samples will be moved to a backup freezer until the primary freezer is repaired. In the event that samples are moved to a backup freezer, the backup electronic recording datalogger will remain with the samples at all times and therefore will also be moved to the backup freezer. If temperatures have exceeded protocol specifications, the study director for those projects affected will be immediately notified of the event and provided with a completed protocol deviation form.

Emergency Backup Freezers:

A. **On-site option:** University of Wisconsin Horticulture Farm, W6797 Kampen Road, Arlington WI 53911. Freezer D (bldg. 916) – This freezer is immediately adjacent to the IR-4 residue sample storage Freezer C.

B. **Off-site option:** University of Wisconsin Arlington Agricultural Research Station, Agronomy, N695 Hopkins Road, Arlington WI 53911. Agronomy Freezer (bldg. 981) – This freezer is located 2 miles away from the residue sample storage Freezer C.

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SOP #22. Residue sample shipping procedures.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that residue samples are removed from storage and shipped to the residue lab with a minimum loss of integrity.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Samples will be boxed and shipped according to the study protocol. Samples will be shipped to the target laboratory as soon as practically possible per protocol instructions.
2. A completed copy of the Residue Sample Chain of Custody Form and a blank Sample Arrival Check Sheet will be included with the samples in each box.
3. Each carton will be labeled with the following information:
 - a. Return name and address of the sender.
 - b. Name and address of the residue lab receiving the samples.
 - c. Bill of Lading Number (if appropriate)
 - d. Field ID Number
4. Untreated samples and treated samples will be packed in separate cartons, and if practical, such cartons will weigh less than 70 pounds.
5. Each carton will be securely sealed with tape. Residue samples will only be out of the freezer long enough to place in prepared box and seal the box with tape.
6. This program will use a freezer motor carrier for transport if possible. If necessary, samples may be shipped utilizing via overnight carrier on dry ice. In the event of overnight shipment, the protocol, study director or IR-4 advisory will be consulted for guidance.
7. The Study Director, Regional Field Coordinator and receiving lab, will be notified of sample shipment.

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SOP #23. Data completion, archiving, retention and quality control review.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that forms are completed accurately and properly document the results of the study.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Any paper forms will be filled out using indelible ink with legible writing or be computer generated. Mistakes on paper forms should be lined out with a single line, initialed, dated, and a reason given for the change.
2. Forms will be filled in completely and accurately as it pertains to the study after the information is available. Sufficient detail or appropriate reference will be provided as to the data collection methods so that they can be repeated if necessary, by a third party with reasonable knowledge of conducting a field trial.
3. The narrative portion of the forms will be used to provide additional detail, summarize the findings or to explain any unusual results.
4. Each study will use existing forms or modifications thereof or develop new forms as needed to conform to the study protocol.
5. Where information is applicable to two or more studies, the original raw data may be placed in one field data notebook and certified copies with reference back to the original placed in the other field data notebooks.
6. All originals will be included in the field data notebook and forwarded to the Regional Coordinator or IR-4 HQ following completion of the field phase of the study. The Field Research Director will retain a copy (paper or electronic) of the field data notebook for their files. Any original data not included in field data notebooks will be sent separately to IR-4 Headquarters for archiving. Data sent for archiving will include an inventory sheet listing the IR-4 test site location, the items being sent in for archiving and the number of pages. The current SOP will be sent to the IR-4 Regional Coordinator for approval and archiving on an annual basis.
7. The Regional Coordinator will review the information received in #6 above for completeness and accuracy in reporting. The regional field coordinator or designee will follow up to obtain any missing data or correct deficiencies with the Field Research Director's consent.
8. The Field Research Director or designee will add any additional or changed pages to the field data notebook copy on file and these updated pages will be used for all subsequent quality assurance audits.
9. Copies of the completed field data notebooks will be retained by the Field Research Director until IR-4 Headquarters advises that these copies may be disposed of.

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SOP #24. Reception, storage and disposal of the test substance.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure the integrity of the test substance.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. IR-4 will be responsible for obtaining a sufficient quantity of the test substance to meet the specifications of the protocol and in obtaining a characterization report (certificate of analysis) on the test substance.
2. Upon arrival of the test substance, receipt in the IR-4 field data notebook will be completed and test substance container will be checked for all GLP required information. Each GLP test substance received will be assigned a unique inventory number that incorporates the trial year for which the test substance is received and a sequential number of receipt for that year.
3. A separate use log sheet will be maintained in the field data notebook for each test substance.
4. All IR-4 test substances will be kept in a locked storage at the University of Wisconsin Horticulture Research Farm, W6797 Kampen Road, Arlington WI 53911 (bldg. 921). This storage will be locked with access only to authorized study personnel.
5. An electronic recording datalogger will be utilized to monitor temperatures in the IR-4 pesticide storage. Temperature data generated by the datalogger will be entered in the field data notebook as raw data. Temperature files stored in the datalogger will be downloaded regularly (approximately monthly) during GLP pesticide storage (less frequently when no GLP test substances are in the storage). A backup electronic recording datalogger will be deployed with the primary datalogger. Data from the backup datalogger will only be downloaded and printed in the event that the primary datalogger fails. If the primary electronic recording datalogger fails, the data from the backup datalogger will be included in the field data notebooks.
6. All test substance containers will be retained by the Field Research Director until approval has been received from IR-4 headquarters allowing disposal. At that time, the containers will either be returned to the sponsor or disposed of in an acceptable manner. The test substances may be used by the facility following the field phase of the study as long as the containers are retained.

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SOP #25. Operation of the mint still and electric boiler.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure the safe and proper operation of the mint still and electric boiler.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: Every time the electric boiler is operated, that operation shall be documented in the Mint Still Log. This boiler will also be maintained and inspected by a qualified boiler inspector according to State of Wisconsin rules and regulations.

1. Inspect the boiler and the still for obvious deficiencies to include such items as leaks or broken parts and general cleanliness. Make sure all steam lines are turned off before proceeding.
2. Turn on the valve controlling the de-ionized water supply for the boiler.
3. Turn on the main power supply for the boiler located on the wall behind the boiler. Then turn on the power switch on the boiler's control panel.
4. Turn on the water supply to the condensers of the still.
5. Remove the mint tub from the stand, remove the top and make sure the inside is clean and the false bottom is in place. Secure top and place tub back on the rack. Drain steam hose by slightly opening valve (using caution to prevent steam burns) and then hook hose to tub and top to condenser with the provided quick connectors.
6. Operate the still empty for five minutes prior to distilling any mint samples to clear the condensers. Also operate the still empty for five minutes between treatments within a study.
7. The check sample will always be distilled first, followed by treated samples from low to high application rates.
8. Fill the tub with the mint hay sample to be distilled, packing it tight by hand. Be certain to fill all corners. Entering steam must permeate the mint hay, not bypass it.
9. Replace the cover on the tub and secure it with the provided clamps.
10. Place the tub on the still's rack and connect the cover to the condenser via the provided quick connector.
11. Place a clean 500 ml separatory funnel in the ring stand under the condenser outlet. Fill the funnel ½ full of deionized water and place a glass funnel in the neck of the separatory funnel to catch the distillate.
12. When the gauge on the boiler reaches 65 psi turn on the main steam valve.
13. Connect the flexible steam hose to the mint tub after carefully draining hose of water.
14. Slowly turn on the steam to the mint tub.

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IR-4 Research Center**

SOP #25. Operation of the mint still and electric boiler, *continued*.

15. When the distillate begins to flow from the condenser, adjust the distillate temperature by controlling the flow of steam into the mint tub. Monitor the distillate temperature by means of a lab thermometer placed in the flow. Maintain a distillate temperature as close as possible to 110°F (43° C). Steam pressure should never be allowed to exceed 3-4 pounds in the mint tub and can be monitored with the pressure gauge on the cover.

16. Maintain the water level in the separatory funnel, draining excess water out the bottom as the distillate enters the funnel.

17. Monitor the flow of mint oil into the separatory funnel. When the oil entering the funnel ceases to flow (after about 10 to 20 minutes), turn off the steam supply to the mint tub.

18. Drain the excess water from the bottom of the separatory funnel (the oil floats on top of the water). When the oil reaches the bottom of the separatory funnel drain the oil into a clean glass sample bottle labeled with the correct treatment information using indelible ink. Line the bottle's cap with aluminum foil before placing it on the bottle.

19. Using extreme caution, remove the flexible steam line from the mint tub, drain water from the tub, disconnect the distillate line and remove the clamps from the cover.

20. Empty the mint tub and clean the inside thoroughly.

21. Repeat steps 6 through 19 for additional mint samples.

22. Each mint sample distilled should be documented on the Mint Still Use Log sheet which is to be placed in the field data notebook.

22. When finished, turn off the power switch on the control panel and the water supply to the boiler. Drain the boiler using the valve located under the McDonnell control valves. When drained, close the valve, turn on the power and the water supply to the boiler. When the boiler has refilled with water, turn off all power to the boiler and turn off the boiler's water supply.

Standard Operating Procedure

University of Wisconsin

IR-4 Research Center

SOP #26. Equipment Maintenance

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that equipment used for carrying out field and greenhouse studies is safe and in proper working order.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Prior to the use of the equipment, the Field Research Director or his designee will visually inspect the equipment to see that it is in good working order, properly lubricated and in reasonably good mechanical condition.
2. Any necessary repairs or adjustments should be made prior to use of the equipment in the trial.
3. The operator of the equipment should be familiar with its operation and safety precautions.
4. Maintenance logs will be filled out for equipment regularly used in a GLP trial. The record should contain maintenance service and repair dates, the operation performed, and whether it was routine or non-routine maintenance. Routine maintenance is defined as expected repairs or normal preventative actions to equipment to keep them in proper working order. Non-routine maintenance is defined as actions taken to repair either unexpected equipment malfunctions or repairs that happen sporadically rather than routinely. Retiring of equipment will be listed as non-routine on its maintenance log and a new log will be established for equipment replacing it or other new equipment. These logs will be entered as raw data in the field data notebook.

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SOP #27. Designated personnel for maintenance and calibration.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that personnel are designated for the maintenance and calibration of equipment as required under the GLP regulations.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. The following person(s) are designated as responsible for maintenance and/or calibration of the following equipment:

- a. Balance (SOP 8): field research directors
- b. Sprayers (SOP 6 &7): field research directors
- c. Weather Instruments and Electronic Recording Dataloggers (SOP 5): field research directors
- d. Freezer and Pesticide Storage: field research directors

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SOP #28. Quality Assurance Inspections.

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel during a quality assurance inspection by the IR-4 QAU.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. IR-4 QA will contact the field research director to make arrangements for the impending inspection.
2. The Field Research Director should notify personnel of the inspection as soon as possible and arrange to make available anyone involved in the trial or facilities audit.
3. The Field Research Director should make certain that all records pertaining to the study/facility inspection are available. This would include:
 - a. Current SOP and study protocols.
 - b. All raw data and logs.
 - c. Training records and cv's of personnel who contributed to the trial.
 - d. Appropriate chain of custody documents for samples, freezer logs and freezer temperature documentation.
 - e. Documentation of the characterization of the test substance, receipt and handling.
 - f. Calibration logs on equipment such as balances and application equipment.
 - g. Storage of records and data associated with the active trials at the location.
4. Have organizational charts and map of the facility available.

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SOP #29. **Procedures to follow prior to, during, and following an EPA inspection.**

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel in the event of an EPA audit or review by the Office of Compliance Monitoring (OCM).

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Prior to inspection (the following procedures will be followed if possible)

1. Notify the Study Director and other personnel of the pending audit or review as soon as possible.
2. Arrange to have available the personnel who may be associated with the trial or facilities audit.
3. Make sure someone who is authorized to accept the Notice of Inspection is going to be present at the start and the finish of the inspection.
4. Prepare study and/or facilities personnel for the inspection.
5. Discuss position descriptions with technical personnel so they understand and can explain their role in the trial.
6. Discuss possible questions that may likely come up about the study or facility
7. Request personnel to answer the questions only to the extent needed and not to provide extraneous information.
8. Make certain that technical personnel know the safety precautions needed for the work area.
9. Be certain that all documents pertaining to the study/facilities inspection are available.

During the inspection (the following procedures will be followed if possible)

1. Greet the inspection team, and escort the entire group to a 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 study. 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 smoother.
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. Proceed with the inspection. Keep notes and or tapes of observations and interviews.

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SOP #29. Procedures to follow prior to, during, and following an EPA inspection (*continued*).

Following the inspection (the following procedures will be followed if possible)

1. Make sure all personnel involved in the inspection are present for the closeout conference.
2. If the inspector's comments are in error, call this to the inspector's attention. Remember, the close out conference is not the forum for any debate.
3. If you have corrected any problems during the inspection, make sure the corrections are noted in the inspector's logbook.
4. Have someone present during the closeout take accurate notes or record the conference on tape if taping is acceptable to the inspectors.
5. Be sure to 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.

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SOP #30. Labeling, handling and storage of spray adjuvants

Written By: D.J. Heider, and S.A. Chapman.

Purpose: To provide guidance to trial personnel using adjuvants in test substance applications

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. All adjuvants used in GLP trials will be labeled with name, concentration, storage conditions, date of receipt, and expiration date. If information regarding is missing for any of these labeling requirements it will be documented as missing or incomplete information in the GLP compliance statement in the field data notebook. It is also suggested that the date an adjuvant container is opened be entered on the container as a helpful reference date. If an expiration date is not available on the label or material safety data sheet (MSDS), then the field research director may assign an expiration date not to exceed 5 years from the date of purchase.
2. Secondary containers (e.g. a 1-gallon container subdivided into several small containers for ease of use and transport to remote sites) are permitted for storage. Secondary containers must be properly labeled as per the original container and now take on all the requirements and properties of an "original container". If temporary containers (a subsample dispensed from a purchased or properly labeled secondary container), they should be used only for the purpose of preventing contamination during measuring. They should be adequately labeled to ensure the product is uniquely identified, but need not be labeled per GLP as required for original or secondary containers. Excess material poured into a temporary container should not be used for subsequent GLP trials and should be discarded.
3. Spray additives will be stored in the IR-4 test substance storage in bldg. 921 at the University of Wisconsin Horticulture Farm, W6797 Kampen Road, Arlington WI 53911. The storage is locked, temperature controlled and monitored as per the GLP requirements for test substance storage.
4. Spray additives will be in good condition prior to use. The physical characteristics will not have changed since purchase or be compromised (i.e. different color, consistency, smell or appear rancid). Document in the field data notebook whether spray additive condition and storage requirements have been met
5. Spray additives will be handled in a way to prevent cross contamination with test substances and other spray additives through either:
 - a. Dispensing spray additives into a temporary container prior to being used in a GLP trial. Any left-over spray additive in the temporary container will not be returned to the original or secondary container, instead it will be disposed of.
 - b. Spray additives will be dispensed from original or secondary containers only using a newly opened pipette or syringe which is discarded immediately after use. The pipette or syringe never returns to the spray additive container and no left-over spray additive is ever returned into the spray additive container.
6. If an earlier purchased spray additive which has not been monitored or labeled to GLP requirements is to be moved into GLP use it must meet the following:
 - a. It must never have been dispensed with a measuring device that has contacted test substance or any pesticide tank mix (i.e. it must not be contaminated)
 - b. It will be treated henceforth as a GLP reagent and points 1 through 5 above will now apply.

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SOP #31. Calibration of backpack mist blower sprayer.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To determine the delivery rate of the plot sprayer and adjust as necessary to ensure an accurate application of the pesticide.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures: To be performed at the beginning of each spray season and within one day prior to application of a pesticide to a residue plot. This procedure must be performed by the person who is going to apply the pesticide.

1. Visually inspect hoses, pipes, fittings and tank for wear, cleanliness and potential leaks. Check fuel and start engine to verify the gasoline engine is operational. At operator's discretion, water may be added to the tank to assist in checking for leaks and verifying that sprayer is operational. Repair or replace any failing components as necessary and record in the sprayer maintenance log.
2. Calibration is performed in the following manner.
 - a. Using a metronome and stopwatch to maintain even pace, sidestep through a known distance moving one foot on each metronome beat and moving the mist blower spray boom up or down the entire height of the plants to be sprayed on each beat. Record the time it takes to complete the movement through the known distance at a constant pace on the calibration form and calculate speed (**ft/sec**).
 - b. Fill sprayer with a known amount of clean water. Set the adjustable exit orifice to the desired setting and holding the boom in a downward position, engage the boom to remove air from the delivery lines. Disengage the boom just as the water reaches the exit orifice (visible through clear delivery lines).
 - c. Start sprayer engine and allow engine to warm up prior to spraying. When engine is warm, engage throttle at full speed and then simultaneously engage the boom and stopwatch. As stopwatch reaches the desired operation time, disengage the boom and then turn off engine. Drain the remaining water from the sprayer into a vessel by holding the boom in a downward position and engaging the boom into the vessel until sprayer is drained. Measure the water drained from the sprayer and the difference between the starting and ending amounts will be the output for amount of time sprayer was operated. Determine the output in **ml/sec**.
 - d. Multiple runs should be within +/-5% of the mean output of the runs to be considered an acceptable calibration. If the calibration is not deemed acceptable, the sprayer should be inspected, repaired if necessary and calibrated again.
 - e. If calibration is to be calculated by hand, determine speed (feet/second) and total output (ml/second) and use these values in the following formula (modified or alternative formula's may be used at the discretion of the field research director) to determine GPA. Record all information and calculations in the field data notebook. The effective nozzle spacing is often the row width or ½ of the row width if sprayed from both sides.

$$\text{Delivery Rate (GPA)} = \frac{\text{Nozzle Output (ml/sec)}}{1} \times \frac{1}{\text{Speed (ft/sec)}} \times \frac{1}{\text{Effective Nozzle Spacing (ft)}} \times \frac{43560 \text{ ft}^2}{\text{Acre Area (A)}} \times \frac{1 \text{ Gal}}{3785 \text{ ml}}$$

- f. To attain changes in output, vary adjustable orifice or speed of travel and recalibrate.

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SOP #32. Hop cone drying procedure.

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that hop cones are properly dried prior to residue sample collection

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Consult the study protocol for guidance on drying temperatures and final desired moisture content of the dried hop cones. If unspecified in the protocol, the drier will be set to 140°F and samples will be dried to between 8 – 12% moisture. When possible dry untreated samples prior to treated samples or place in separate dryers.

2. Wet hop cone samples will be collected into labeled mesh bags which will be placed in the forced air drier in a relatively consistent depth to facilitate even drying of cones. Cones will be selected randomly from the samples for both dry matter determination and to monitor the drying progress as described below.

3. Dry matter is determined by completely drying a small subsample in a microwave oven. The wet weight of the subsample will generally be between 50 and 150 g. The subsample of cones will be spread out on a paper plate or other suitable substance and microwaved on high power until fully dry (the point at which the weight of the cones no longer decreases while microwaving).

$$\% \text{ Dry Matter} = \frac{\text{hop cone dry weight}}{\text{hop cone wet weight}} \times 100$$

4. A subsample of generally 50-150 g is placed in a cotton bag (a finer mesh to prevent loss of any hop cone fragments during the drying and continual weighing to monitor the drying process). This subsample is weighed wet and placed into the drier with the larger samples for drying.

$$\text{Subsample cone wet weight} = (\text{weight of cones} + \text{bag}) - \text{bag weight}$$

5. Determine lower and upper target weights of the dry subsample to assess when the hop cones have reached the target range (the following formulas use 8-12% moisture but can be adjusted as needed).

$$8\% \text{ moisture target weight} = \frac{(\% \text{ dry matter} \times \text{subsample wet weight}) + \text{bag weight}}{92}$$

$$12\% \text{ moisture target weight} = \frac{(\% \text{ dry matter} \times \text{subsample wet weight}) + \text{bag weight}}{88}$$

6. The cotton bag subsample weight should be monitored during drying with decreasing intervals between checks as the hop cones approach the target weight range. Continue drying hop cones until the cotton bag subsample weight is within the target range. Drying time generally takes between 6 and 10 hours and is dependent upon the sample size, original moisture content and ambient humidity of the air. When dry, allow the hop cones to equilibrate to room temperature (generally not longer than 1 hour), mix the cones and collect random handfuls from each sample until the desired amount of residue sample is obtained.

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SOP #33. Use of borrowed or seldom used equipment in GLP studies

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that borrowed equipment is operated and cleaned appropriately in the conduct of GLP trials.

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

1. Prior to use, ensure that any available owners/operation manual(s) are reviewed and understood.
2. If borrowed equipment lacks maintenance and/or use logs, document all actions relative to the piece of equipment in the field data book (including, but not limited to: any maintenance actions while in your possession, use description and cleaning if needed).
3. Visually inspect the equipment to assess that it is in good working order. Any problems discovered prior to use should be corrected and documented. If the equipment has the potential to transfer chemical residues to the test system crop, then prior to use and between treatments clean the equipment surfaces which may contact the crop.
4. Perform the required tasks with the equipment. Document/describe the use in the field data book.
5. After completing the tasks, clean surfaces of the equipment that may have contacted the treated test system crop prior to returning or storing the equipment.
6. In the rare event that borrowed equipment is used in the generation, measurement or assessment of data, it will be adequately tested, calibrated and/or standardized prior to use and these actions will be properly documented in the field data book.

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SOP #34. Operation, Maintenance and Standardization of Geode Global Positioning System

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that GPS unit used in the conduct of GLP trials is in good working order

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

Operation:

1. Prior to first use, ensure that any available owners/operation manual(s) are reviewed and understood. Charge GPS receiver/antennae as needed to ensure power when using in the field
2. Power up the GPS unit and connect via Bluetooth or cable to a device utilizing an app that allows readout of the GPS unit. Placing device in airplane mode will assure disablement of the internal GPS, allowing the external Geode GPS to become the sole source of GPS.
3. When the GPS unit (through the connected device app) indicates a Position Dilution of Precision (PDOP) of less than 3 or the estimated horizontal precision is less than 1.0 m, record a waypoint while holding the GPS receiver/antennae directly over the position to be recorded. If this position is a plot corner, record the latitude, longitude and indicate if estimated horizontal precision was less than 1.0.
4. To navigate back to a point, place connected device into airplane mode to disable its internal GPS. Power up the GPS unit and connect to the device via Bluetooth or cable. Select a familiar mapping app and enter the latitude and longitude of the point of interest. Follow the on-screen visualization of your movement until the GPS cursor and the point of interest overlap one another on the screen.
4. After completion of waypoint collection or navigation, power down the GPS unit.

Maintenance:

1. The only maintenance is to charge the GPS receiver/antennae battery utilizing the charger provided with the unit.

Standardization:

1. Select a location with known coordinates. This may include a permanent location that is visible in satellite view in Google Maps or another mapping application. Utilizing the GPS receiver/antenna record two separate waypoints at the location with the known coordinates.
2. At the beginning of each growing season compare the known coordinates with those recorded by the GPS unit (record both in the GPS log). Each degree of latitude is equal to 111,092.7 meters. Each degree of longitude at 43° latitude is equal to 81,540.9 meters. If the difference between the known and recorded coordinates is greater than 1.0 meter for either latitude or longitude, then re-standardize the unit with two new recorded waypoints. If the difference is still greater than 1.0 meter, then repair or replace the GPS unit.

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SOP #35. Testing for THC content in Hemp Studies

Written By: D.J. Heider and S.A. Chapman.

Purpose: To assure that THC of harvested hemp is below 0.3%

Scope: All field studies conducted under Good Laboratory Practices (GLP's).

Procedures:

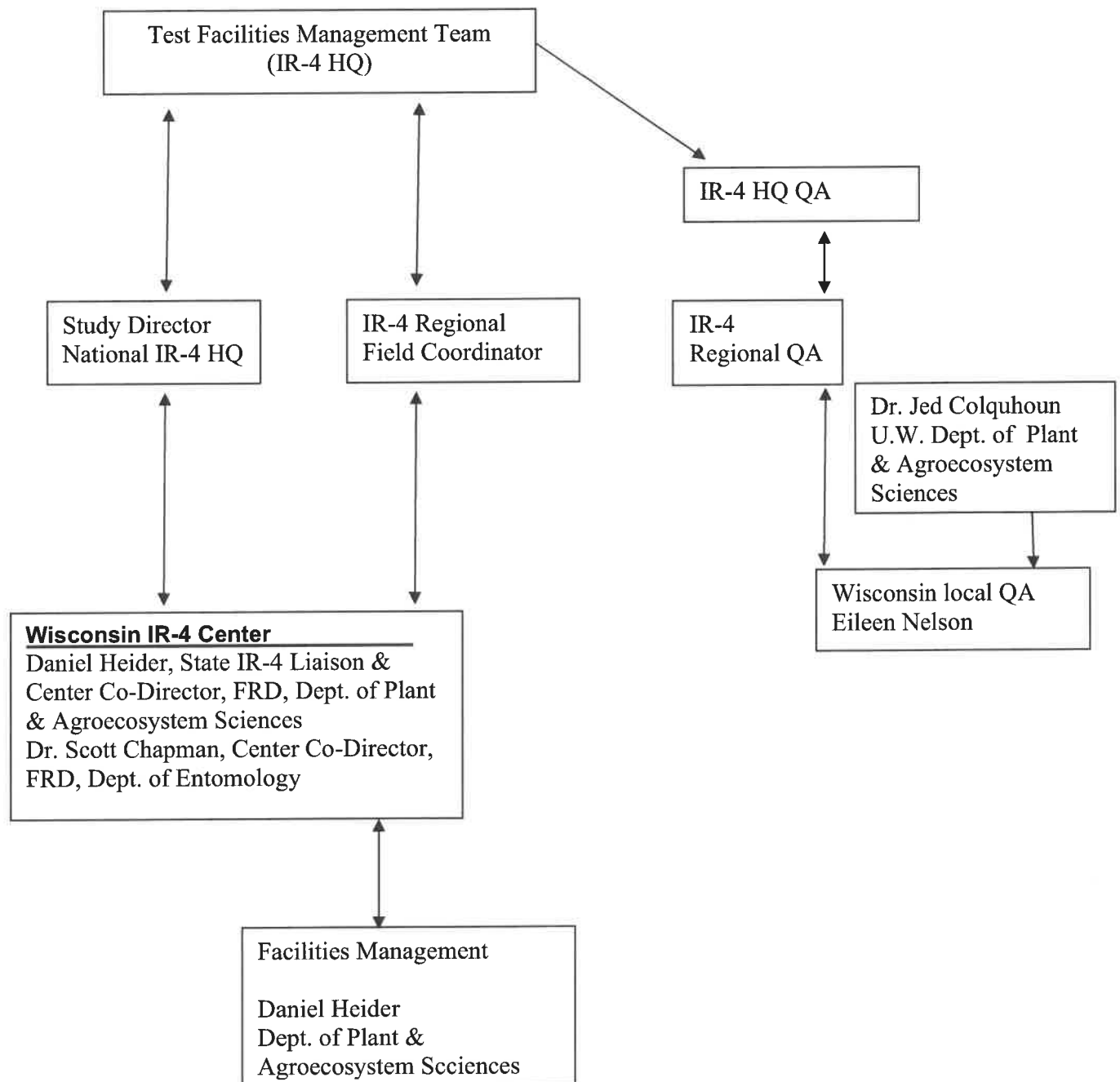
1. Each lot of hemp shall be sampled for total delta-9 THC prior to harvest. A lot of hemp is defined as a contiguous area in a field, greenhouse, facility, or growing structure containing the same variety or strain and age of hemp throughout the area. Two or more field trials (including residue studies) may be sampled as a single lot if they meet the criteria.
2. Within a 30-day period prior to harvest, collect shoot tips (including all plant parts) from a specified number of plants for each lot. Consult the protocol for specific requirements on length of shoot tips to be sampled. It may be appropriate to sample additional plants if lot size is very large or lacks uniformity in growth.
3. The collected shoots should be harvested into a paper bag labeled with study ID number and treatment number.
4. The bagged shoots should be placed in a protected area to facilitate drying until transport to the testing lab which should occur as soon as reasonably possible. Depending upon the lab requirements, the shoots may be dried fully before transferring to the lab or taken to the lab where additional drying may happen prior to testing.
5. A University of Wisconsin research lab or private industry contracting lab may be chosen for THC testing. Only reputable labs with documented analytical methodology will be chosen for THC testing.
6. Results of the THC test should be received prior to shipping any hemp residue samples. If THC is greater than 0.3%, the crop (including any harvested samples) will be destroyed, and the study director will be notified. If testing confirms THC content of 0.3% or less, the testing report will be included in the field data notebook and a copy will be sent with the residue sample shipment.

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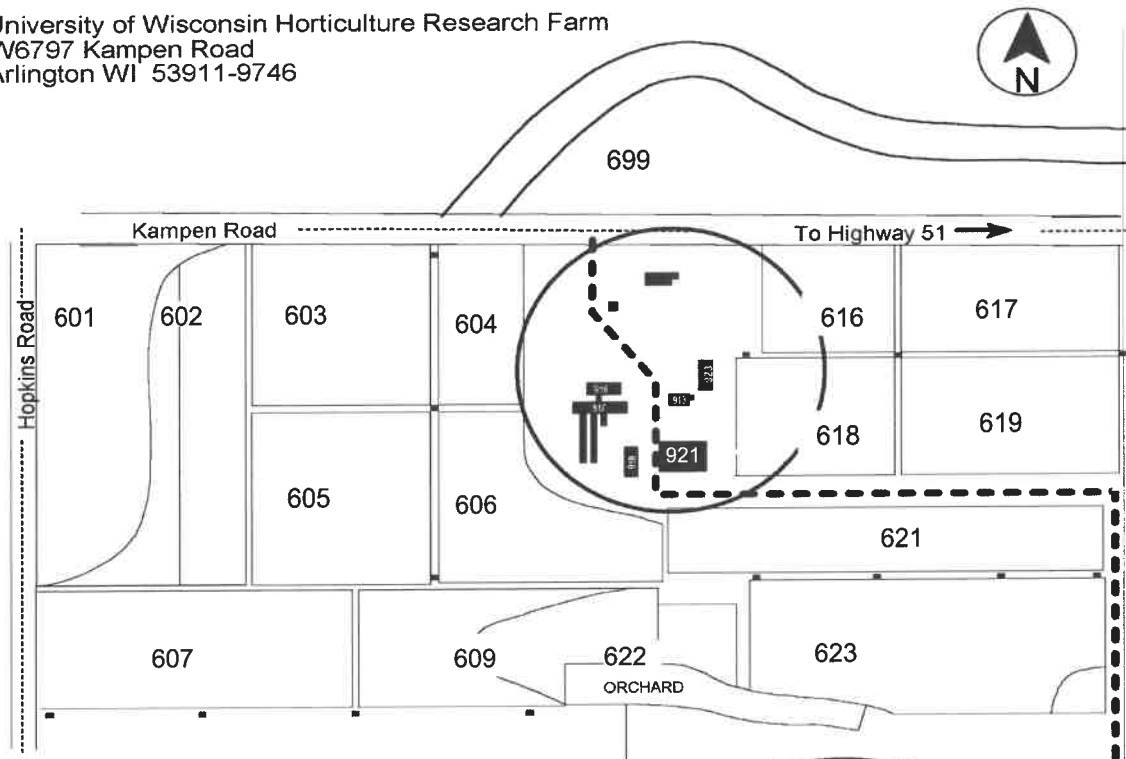
Appendix A. Personnel Flow Chart, Wisconsin IR-4 Center



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Appendix B. IR-4 Center Facilities, Horticulture Research Farm, Arlington, WI.

University of Wisconsin Horticulture Research Farm
W6797 Kampen Road
Arlington WI 53911-9746

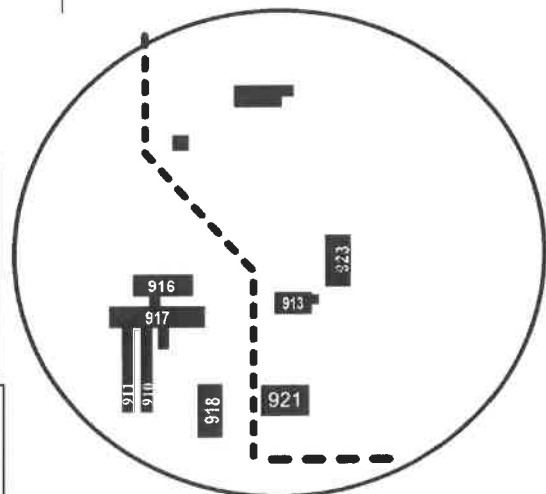


KEY

Bldg 913 - Storage
Bldg 916 - Lab and office, Freezer storage
Bldg 917 - Headhouse, Mint still
Bldg 918 - Tobacco shed
Bldg 921 - Ag chemical storage, CO2 sprayer storage
Bldg 923 - Shop and machine shed
--- = Farm driveway
■ = permanent field marker

Additional Information:

Bldg 910 Greenhouse (aka. Hort Greenhouse)
Bldg 911 Greenhouse (aka. USDA Greenhouse)
Un-numbered short greenhouse east of bldg. 910 is "stub-house"
Bldg 916 Office contains locked fireproof FDB storage
Bldg 917 is the headhouse to 3 greenhouse ranges & residue sample bag storage
Bldg 921 also contains the GLP Balance and contains the entry into the test substance storage



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Appendix B. IR-4 Center Facilities, Entomology Research Farm, State Highway 51, Arlington, WI.

County: Columbia
Township: Leeds
Section: 20

UW - Arlington Agricultural Research Station

500 Fields

KJM 3/25/2015

