AGRICULTURE AND AGRI-FOOD CANADA (AAFC)
DISLODGEABLE FOLIAR RESIDUE DISSIPATION STUDY PLAN

ACTIVE INGREDIENT:
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES

STUDY #: AAFCXX-XXXD

STUDY DIRECTOR:

AAFC Minor Use Pesticide Program
Building 57, Central Experimental Farm
960 Carling Avenue
Ottawa, Ontario K1A 0C6
Phone:
Fax:
Email:

FIELD TRIAL LOCATION:

Refer to Section 10, page 3.

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1. STUDY TITLE:
Active Ingredient: Dislodgeable Foliar Residue Dissipation from Greenhouse Tomatoes

2. JUSTIFICATION AND OBJECTIVES:
Agriculture and Agri-Food Canada (AAFC) has received a request for the minor use label expansion of *Active Ingredient* on greenhouse tomatoes. A Dislodgeable Foliar Residue (DFR) study is to be conducted according to support an exposure and risk assessment for workers re-entering greenhouses treated with Active Ingredient, as per Regulatory Proposal 98-04 (September 4, 1998) *Postapplication Exposure Monitoring Test Guidelines*. The purpose of this study is to determine the residues of Active Ingredient that can be dislodged from greenhouse tomato foliage, to determine the potential of re-entry exposure. This study plan will be implemented using applicable Standard Operating Procedures (SOPs) and conducted under provisions outlined in Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practices (GLP) (1997 Revision). Any work conducted in the USA will be conducted according to Environmental Protection Agency (EPA) Good Laboratory Practice standards, 40 CFR part 160, which are acceptable to OECD standards.

3. SPONSOR/TESTING FACILITY NAME, ADDRESS AND PHONE:
AAFC Minor Use Pesticide Program, Building 57, Central Experimental Farm, 960 Carling Avenue, Ottawa, ON, K1A 0C6, Phone: (613) 715-5390, Fax: (613) 694-2323

4. STUDY DIRECTOR:
Name, Building 57, Central Experimental Farm, 960 Carling Avenue, Ottawa, ON, K1A 0C6, Phone: Fax: Email:

5. COMPLIANCE:
The test facility and appropriate test sites (field and laboratory) will be responsible for certifying that its portion of the study will be conducted in accordance with the OECD Principles of GLP (1997 Revision). Any work conducted in the USA will be conducted according to EPA Good Laboratory Practice standards, 40 CFR part 160, which are acceptable to OECD standards. A statement of compliance, together with any deviations will be signed and submitted by the responsible Study Director in the Final Report and by the Principal Investigator in their Raw Data Field Notebook (RDFN) or analytical report.

6. QUALITY ASSURANCE:
Quality Assurance (QA) duties and responsibilities will be in conformance with the OECD Principles of GLP (1997 Revision). Any work conducted in the USA will be conducted according to EPA Good Laboratory Practice standards, 40 CFR part 160, which are acceptable to OECD standards. A Quality Assurance Statement will be provided by the QA for each site, for each Raw Data Field Notebook, Final Analytical Report and Final Report. It shall include the type of inspections, the date inspections were made and date(s) any findings were reported to the Study Director, Principal Investigator (if applicable), and management(s).

7. TEST FACILITY RECORD KEEPING:
A study file will be initiated and maintained by the Test Facility. Original study plan, amendment(s), and deviation(s) if any, as well as the original raw data (e.g. RDFNs, laboratory data [each of which may contain copies of facility records]), final analytical report and final report will be archived by the Test Facility.

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8. PROPOSED DATES:
Experimental Start:
Experimental Termination:

9. STUDY SIGNATURES:

Study Director/ Date

AAFC Minor Use Pesticide Program
Building 57, Central Experimental Farm
960 Carling Avenue
Ottawa, ON K1A 0C6
Phone:
Fax:
Email:

Quality Assurance/ Date

AAFC Minor Use Pesticide Program
Building 57, Central Experimental Farm
960 Carling Avenue
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Phone:
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Email:

Test Facility Management/ Sponsor Representative/ Date
Submissions Manager
AAFC Minor Use Pesticide Program
Building 57, Central Experimental Farm
960 Carling Avenue
Ottawa, ON K1A 0C6
Phone:
Fax:
Email:

10. FIELD (GREENHOUSE) PERSONNEL/TRIAL ID NO:
(Responsible for Sections 11-25)
The Principal Investigator and Site Management must sign and return the attached GLP acceptance form (see Appendix A) for each Trial ID #.

PRINCIPAL INVESTIGATOR: **TRIAL ID No.**

TEST SITE MANAGEMENT:

11. TEST SYSTEM/CROP:
Greenhouse tomato - use a commercially cultivated variety. At a minimum, record the variety, and age of planting, if available. **Plants** need to be of **adequate size** to ensure there is enough foliage for multiple sampling over the entire trial and a **leaf surface area** large enough to ensure **complete discs** are obtained during sampling. This study will take place at a designated site to provide useful DFR data for this crop.

12. TEST SYSTEM DESIGN and STATISTICAL METHOD:
The trial site will consist of an untreated control plot and a treated plot. The test system should represent or simulate the major greenhouse production house design used by area commercial growers. The individual plots shall be of adequate size (a minimum of X plants) to allow collection of all necessary plant material without sampling border rows, plot ends or adjacent plants on any given sampling date. Consideration should be given to the growing space provided the plants to ensure that individual plant leaves can be easily identified to the plant throughout the sampling schedule.

At a minimum, a 5 m buffer zone will be employed around the untreated and treated plots to prevent contamination[1]. **Alternatives to using a minimum 5 metre buffer may include: a physical barrier separating the untreated and treated plots in the greenhouse to prevent contamination; untreated and treated plots can be placed in separate greenhouses or separate bays within the same greenhouse.** Mark plots with identifiable markers containing at a minimum the Trial ID # (AAFCXX-XXXD-XXX), and treatment number or treatment name that will persist for the duration of the field research trial or that can be readily replaced. A plot map enabling trial site relocation by a third party must be created. This study is not designed for the statistical evaluation of field data.

13. TRIAL SITE PREPARATION AND MAINTENANCE:
Prepare or select a trial site that has been and will continue to be maintained following local, good agricultural practices for the production of greenhouse tomatoes, including fertilization, irrigation and other practices that ensure good greenhouse tomato production. **Sprinkler (overhead) irrigation is prohibited in this study. Drip irrigation, sub-irrigation or some similar method, is allowed.**
The trial site cannot have been treated with a chemical similar in nature to the test item (as outlined in section 17) during the life cycle of the crop. The trial site will have a known pesticide history of a minimum of the life cycle of the crop (provided greenhouse was thoroughly cleaned and previous crop disposed of prior to trial initiation). The trial site must be maintained throughout the season to ensure that weeds do not interfere with treatment applications or crop maturity. Note: A soil analysis (≤ 5 years old) must be provided for the trial (see section 23 for details). If an artificial medium is used, provide a detailed description of its composition in place of a soil analysis.

[1] Note that the buffer zone is considered the area outside of the plot and the spray zone that is a conflicting chemistry and test item free zone. The spray zone is an area before and after the treated plot that allows the operator to activate the boom and get up to the appropriate speed before entering the plot; and it is an area after the plot that allows the application to continue outside of the treated plot; which helps to ensure a uniform coverage to the treated plot. It is also an area where the boom can be primed and discharged, as long as contamination of the plot is avoided.

14. TEST ITEM:

Use the *(Product name)* formulation of Active Ingredient (CAS #:) **that has been characterized to meet GLP** standards. AAFC will arrange procurement of GLP test item from the Registrant. Upon receipt, document the lot/batch number, condition, quantity received and confirmation of GLP characterization. Contact the Study Director if there are any concerns regarding the GLP characterization, label identification of the test item (e.g., the name on the bottle or certificate of analysis (CoA) is different from the study plan), etc. and if the CoA does not come with the test item. Store the test item in a secure, clean, dry area at temperature ranges noted in the product label or other references.

Prior to test item disposal, contact the Study Director for specific instructions. Unless otherwise specified, the registrant will archive a retention sample of the test item.

15. TEST ITEM APPLICATION:

To ensure that the test item is well mixed, agitate during the application, if practical. Observe the test item in the spray mixture and provide documentation in the RDFN that the test item was completely dissolved/ mixed in the carrier before application. Use application equipment that will provide uniform application of the test item in the required spray volume (see section 16). Apply the test item as specified (see section 16), in a manner that represents or simulates the major application technique that is used by area commercial growers. The test item, if applied in a mixture, must be applied to the test system within 2 hours of mixing. The test item must be applied in a manner to ensure accurate delivery and to prevent contamination to adjacent plots. Ensure there is minimal air circulation during the application (i.e. the **fans turned off**).

To ensure accurate delivery, calibration for output and speed must be performed. Just prior[2] to the application of test item, calibrate for nozzle and speed (equipment or walking speed), by performing a minimum of three, consecutive acceptable checks (within ±5% of the average output, or -5% to +10% of the target pass time for speed calibration); or by performing a minimum number of runs for which at least 75% of the total number of checks are acceptable (i.e. 3 acceptable runs out of a total of 4 checks performed. Note in this situation only the values from the 3 acceptable runs will be used for calibration calculation). This is considered a **complete calibration**. Conduct the speed calibration at the edge of the test plot, or on similar terrain. The uncharged spray boom may be held over or directed at the plot.

At a minimum, for multiple applications performed on the same day within a trial or between trials, a single recheck of the output and speed is necessary. A single output check must be conducted to confirm consistent delivery (± 5% of the last complete calibration) just prior to subsequent applications. This is considered a **calibration recheck**. Note: a calibration recheck is only acceptable if application parameters or equipment components have not changed. If the **calibration recheck** results in an output that differs from the mean output of the **complete calibration** by more than ± 5%, then the equipment must be completely re-calibrated.

If application parameters (e.g. application type, water volume) or equipment components (e.g. nozzle tips) have changed from the initial calibration, another **complete calibration** (of nozzle output and/or speed, depending on what was modified) must be performed and documented, even if the equipment has been changed back to the parameters of the initial calibration. (Equipment logs should be used to document changes in the equipment parameters).

[2] "Just prior" includes the day prior to the application, but calibration on the day of use is preferred.

If the complete calibrations were conducted as part of another trial, a true copy of all complete calibrations references along with the required rechecks performed for this trial are to be included in the raw data field notebook. **Calculations for the amount of test item to be applied will always be based upon mean output calculated from the most recent complete nozzle output or speed calibration data, not on the recheck results.**

Record actual application pass times in the RDFN and verify the accuracy of the application. The application is considered acceptable if the accuracy is within -5% and +10% of the study plan specified application rate, surfactant rate and the spray volume range limits. If the application does not meet this range, the Study Director must be notified of this deviation before proceeding with this trial.

Use application methods that result in maximum coverage. Ensure the targeted spray area receives a consistent spray by starting and ending the application before and after the defined plot area, respectively (this includes the plot ends and guard rows that will not be sampled from).

16. APPLICATION TREATMENTS AND TIMING:

Trt#	Treatment	Target Rate of active ingredient	Target Rate of formulated product*	Application Type**	Spray Volume
01	Untreated	Not Applicable	Not Applicable	Not Applicable	Not Applicable
02	Active Ingredient	X g a.i./ha*	X L/ha	Broadcast, foliar	Minimum of 1000 L/ha, spray to runoff

*The nominal formulation concentration of the test item will be used in calculating the final application rate (see section 14 for the nominal concentration).

****Make 4 (Broadcast, foliar) applications at X (±1) day intervals. Sampling will begin one day prior (or on the same day but prior to) the first application**.

Surfactant:

Note: for any other additive to the spray solution (such as but not limited to antifoaming agents or pH adjusters) contact the study director for approval.

17. SUPPLEMENTAL GREENHOUSE TOMATO TREATMENTS:

The integrity of the field trial should be protected by minimizing damage to the test crop caused by pests. Only registered maintenance pesticides applied according to labeled directions can be used, unless approved by the Study Director. Approval from the Study Director to use non-registered pesticides is to be documented in the RDFN. Make identical applications to the untreated and treated plots. Document all supplemental crop treatments. DO NOT USE pesticides which are similar to the test item such as morpholine (Group 5) fungicides, or other chemicals that might interfere with analysis of the test item. If unsure, contact the Study Director.

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18A. RESIDUE SAMPLE COLLECTION (SEE SECTION 21: GREENHOUSE DFR SAMPLE INVENTORY, TABLES 1, 2 AND 3):

Samples will be collected using validated Birkestrand (or equivalent) leaf punch samplers capable of producing a circular leaf disc with a diameter of 2.54 cm (or 1"). A separate leaf punch sampler must be used for each of the untreated and treated plots. The Birkestrand (or equivalent) leaf punch discs need to fit into glass jars which will receive the leaf disc directly upon punching. The jars will be pre-labeled as outlined in Section 18B, prior to collection. All leaf punch discs will be taken when **foliage is dry**, disposable gloves and protective clothing worn, and care taken to not handle any area of the foliage that may be sampled (during the current or future sampling event). For each application date document how long it takes the foliage to dry from the time of application to when sampling begins in the treated plot. Collect the untreated samples prior to the treated samples or use a different person to collect the treated samples.

Each sample will consist of 40 leaf discs (as needed for a sample of approximately 400 cm^2 total leaf surface area, counting both sides of the leaf), unless there is documented approval from the Study Director. Document how the leaf discs were counted and accuracy ensured. The leaf punch will be cleaned with an appropriate solvent(s), after each sample of 40 leaf discs is taken. Record sampling times and cleaning procedures in the RDFN. The forty leaf discs will be collected in a manner to ensure a representative, impartial sample. The procedure should ensure that each sample is made up of representative plant material from all areas of the plot excluding borders and ends. Borders for the plots may consist of plants other than greenhouse tomatoes that are designated for exclusion from sampling and any maintenance applications that include Active Ingredient. **No more than two discs may be collected from any single plant on any sampling date.** Plants may be re-sampled on different sampling dates. Samples will be taken systematically from high, low and middle areas of the plants as well as from all sides of the plant. When collecting leaf discs, care must be taken to ensure that the entire cutting area is covered by plant material (i.e., a full circular disc is collected) otherwise the area of the leaf disc will not be known. In general the leaf disc should be collected near the top of the leaf, but not the tip. **New leaves**, including leaves **not present at the previous application must be avoided**.

Note: A metal tag or permanent markers will be used to mark the section of the plant so that new leaves that grow after the first treatment will not accidently be sampled. Record the sampling procedures used in the RDFN.

At a minimum, twelve (12) samples (Table 1) consisting of 40 leaf discs per sample can be collected from the **untreated** or **treated** plot before application # 1. These samples will be used by the laboratory **for analytical method validation** and **method fortification recoveries** during the analysis of samples for dislodgeable residues. The Study Director will inform the Principal Investigator of the timing for the collection of the untreated samples based on when the lab is ready to begin method validation.

At each sampling interval (Table 2), one sample will be collected from the untreated plot and three samples from the treated plot. Samples will be collected on the following days: 1 day prior or on the same day (but prior to) application #1 (PRE 1); on the day of application #1 (4 ± 1 hours after the first application – leaves must be dry) (POST 1); 3-4 days after application #1 (DAY 3-4, POST 1); 1 day prior or on the same day (but prior to) application #2 (PRE 2); on the day of application #2 (4 ± 1 hours after the second application – leaves must be dry) (POST 2); 1 day prior or on the same day (but prior to) application #3 (PRE 3); on the day of application #3 (4 ± 1 hours after the third application – leaves must be dry) (POST 3); 1 day prior or on the same day (but prior to) application #4 (PRE 4); on the day of application #4 (4 ± 1 hours after the fourth application – leaves must be dry) (POST 4); and 1, 2, 4, 7, 10, 14, 21, 28 and 35 days after application #4 (Day 1, POST 4; Day 2, POST 4; Day 4, POST 4; Day 7, POST 4; Day 10, POST 4; Day 14, POST 4; Day 21, POST 4; Day 28, POST 4, Day 35, POST 4). The untreated samples

listed in Table 2 are the same control samples listed in Table 3 for the same sampling interval. They can also be run as the controls for the field recovery samples.

An additional six samples (consisting of 40 leaf discs per sample) in total will be collected from the **untreated** plot for **field recoveries** (three Low concentration and three High concentration field recoveries) on the day of application #1, the day of application #2, the day of application #3, the day of application #4, and 7, 14, 21, 28 and 35 days after the fourth application (Table 3). Plus a Low concentration fortification vial and High concentration fortification vial will be used as travel recovery samples.

18B. SAMPLE COLLECTION

Identify each jar of leaf samples as follows:

Nature of Study:	DFR Study
Test System/Test Item:	Greenhouse tomato/Active Ingredient
Trial ID Number:	AAFCXX-XXXD-XXX
Sample ID Number:	as per Study Plan Part 21
Treatment Number:	as per Study Plan Part 21
Sample Interval:	as per Study Plan Part 21
Sample Matrix:	as per Study Plan Part 21
Sample Date:	date sampled

19. SAMPLE DISLODGING PROCEDURES:

Containers with leaf discs will be kept cool by placing them in a cooler with ice (blue or wet) for temporary storage prior to dislodging. The samples may be transported from the greenhouse to test site laboratory or other area where the dislodging will take place. Dislodging of the leaf samples will be performed as soon as possible, within **4 hours** after collection. The leaf discs will not be frozen prior to processing.

Samples will be dislodged with 0.01% Aerosol® OT solution (anionic detergent solution) prepared using distilled or de-ionized water. Dilute as needed to achieve a concentration of 0.01 %. The preparation of the Aerosol OT solution will be documented in the RDFN. The shelf life of the Aerosol® OT solution at room temperature is 48 hours. Dislodge the untreated samples prior to the treated samples or use a different person to dislodge the treated samples.

Next, 100 mL of the 0.01% Aerosol® OT solution will be added to each jar containing leaf discs. The jars will be capped securely and placed on a reciprocating shaker operating at approximately 200 revolutions per minute (RPM) for a period of approximately 10 minutes. Sample dislodging time will be documented in RDFN.

After shaking, the leaf discs will be separated from the solution by carefully decanting the entire solution (except for what may adhere to leaf surfaces) into clean labeled (as outlined in Section 18B) glass containers. Any leaf discs transferred to the extract container will be removed with clean tweezers and returned to the original sample jar for the second dislodging. Another 100 mL of the dislodging solution will be added to the leaf discs and the dislodging process repeated (approximately 10 minutes of shaking at approximately 200 RPM). The solution will be decanted into the same container as the first 100 mL of dislodging solution. The leaf discs can be discarded at this stage. **Teflon-lined caps** will be loosely screwed onto the sample jar and sample jars placed **at an angle** in the freezer to minimize the chances of container breakage

during freezing (except those that are to receive fortifications as outlined in Section 20). The **lids** will be **tightened after freezing** has occurred, prior to shipping. All procedures used for sample processing will be documented within the RDFN.

20. FIELD RECOVERY AND TRAVEL RECOVERY SAMPLES (SEE SECTION 21: SAMPLE INVENTORY, TABLE 3):

The stability of the analytes in the extract solution from the time of sampling, to the time of analysis shall be demonstrated by fortifying an untreated sample extract solution with the reference items. This is accomplished by adding a fortification solution to an untreated sample extract solution obtained in the dislodging step (Section 19). On fortification days, six samples of untreated leaf discs (each containing 40 leaf discs) will be collected from the untreated plot for fortification purposes and labeled according to Sections 18B and 21 (Table 3). Field fortifications should be performed in triplicate at two fortification concentrations (Low and High).

Follow the procedures in Section 19 up to and including the discarding of the leaf material. Do not cap and freeze the jars of solution. **Three of the four low concentrations and three of the four high concentration vials will be used for field recovery samples.** Insert one of the pre-measured fortification vials from the laboratory to the remaining jars (See the NOTE at the bottom of this section). Ensure the amount of solution in the vial corresponds to the **horizontal mark** on the vial; if it does not, select another vial to add to the dislodging solution. This vial will be opened, inverted, gently tap the bottom to drain the contents and drop the vial and cap into the dislodging solution. Tighten the Teflon-lined cap and swirl to mix and store frozen as described above in Section 19. Control samples and field recovery samples will be prepared a reasonable distance away from the treatment area to avoid potential contamination.

The **fourth fortification vials** (Low and High concentration) will be used as **travel recovery samples**. Travel recovery samples are utilized to demonstrate the stability of the analytes during shipment and storage. The travel recovery samples should be handled exactly as the vials used for the field recoveries up to but not including the fortification step – after the fortification above, the travel recovery samples should be placed into frozen storage along with the field recovery samples. These vials are **returned to the lab unopened** to be used by the lab as **travel recovery samples**. Keep the travel recovery samples in separate containers from the field samples in the storage unit and when shipping to prevent the possibility of cross contamination. **Teflon-lined caps** will be loosely screwed onto the sample jar and sample jars placed **at an angle** in the freezer to minimize the chances of container breakage during freezing. **After freezing** has occurred the **lids** will be **tightened**. All procedures used to prepare the field recovery and travel recovery samples will be documented in the RDFN. Label travel recovery vials as follows:

Nature of Study:	DFR Study
Test System/Test Item:	Tomato/Active Ingredient
Trial ID Number:	AAFCXX-XXXD-XXX
Sample ID Number:	as per Study Plan Part 21
Date:	date sampled

NOTE: Fortification solutions will be made from the Reference Items by the analytical laboratory identified in Section 26, as specified in Section 27. Vials containing 1.0 mL of each fortification solution will be provided by the laboratory. Vials containing the low concentration will be marked with an “L” and the high concentration vials with an “H”. A **horizontal line** marked on the vial should indicate the amount of solution. The laboratory will prepare 4 vials of each concentration

(low and high) for each fortification day, plus 6 additional contingency vials, for a total of 42 vials per concentration, and ship them to the field site. *Active ingredient* standard solutions are stable for up to XX days, **therefore samples must be analyzed within XX days from the date the fortification solution was prepared**. Shipment of the fortification solutions will be done using recommended storage conditions. The field site will store the vials under the recommended conditions until needed. **Send samples to the laboratory identified in table below, as soon as practical. For samples packed with dry ice, avoid shipments from Thursday through Sunday.**
After shipping all the field and travel recovery samples any unused fortification solution vials may be disposed of according to local regulations.

Trial ID No.	**Ship to: (Trial ID No., Contact and Shipping Address)**
AAFCXX-XXXD-XXX	AAFCXX-XXXD-XXX Attn: Phone: Fax: Email: See section 26 for responsible person for this Trial ID No.

21. GREENHOUSE DFR SAMPLE INVENTORY:

A list of the samples to be collected is presented in Tables 1, 2 and 3. Samples obtained in Table 1 can be collected from the **untreated** or **treated** plot since they will be collected before the Test Item has been applied. These samples will be used by the laboratory for analytical method validation and concurrent fortification recoveries during the analysis of samples for dislodgeable residues. The Study Director will be contacted by the Laboratory when control samples are required. The Study Director will in turn arrange with the Field PI to collect the necessary samples. Samples obtained in Table 2 will be used to determine the dislodgeable foliar residue data. Samples in Table 3 will be fortified with the analytical standard solution to demonstrate the stability of the active ingredient(s) in the leaf - dislodgeable solution matrix over the period of sampling to analysis (field recovery), and include travel recovery samples to verify the integrity of the field recovery samples. The PI will record the date of sampling in the RDFN and assign the appropriate sample ID numbers (V-01, V-02, etc) to the samples. In addition, unused vials from the field fortification (i.e. travel recovery samples) will be handled in the same manner as samples and returned to the analytical laboratory with the fortified samples, to be analyzed as travel recoveries.

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Table 1: Control Samples for Method Validation

SAMPLE ID	SAMPLING INTERVAL[1]	SAMPLE MATRIX
V-01	Prior to the first application	Dislodging Solution
V-02	Prior to the first application	Dislodging Solution
V-03	Prior to the first application	Dislodging Solution
V-04	Prior to the first application	Dislodging Solution
V-05	Prior to the first application	Dislodging Solution
V-06	Prior to the first application	Dislodging Solution
V-07	Prior to the first application	Dislodging Solution
V-08	Prior to the first application	Dislodging Solution
V-09	Prior to the first application	Dislodging Solution
V-10	Prior to the first application	Dislodging Solution
V-11	Prior to the first application	Dislodging Solution
V-12	Prior to the first application	Dislodging Solution

[1] Sampling interval and timing is outlined in Section 18A.

Table 2: Samples for Dislodgeable Foliar Residue Determinations

SAMPLE ID	TRT #	TRT	SAMPLING INTERVAL[1]	TIMING	SAMPLE MATRIX
A1-01	01	Untreated	PRE 1	1 day prior or on the day of (but prior to) application #1	Dislodging Solution
A1-02	02	Active Ingredient	PRE 1	1 day prior or on the day of (but prior to) application #1	Dislodging Solution
A1-03	02	Active Ingredient	PRE 1	1 day prior or on the day of (but prior to) application #1	Dislodging Solution
A1-04	02	Active Ingredient	PRE 1	1 day prior or on the day of (but prior to) application #1	Dislodging Solution
A1-05	01	Untreated	POST 1	day of application #1	Dislodging Solution
A1-06	02	Active Ingredient	POST 1	day of application #1 (4±1 hours after application)	Dislodging Solution
A1-07	02	Active Ingredient	POST 1	day of application #1 (4±1 hours after application)	Dislodging Solution
A1-08	02	Active Ingredient	POST 1	day of application #1 (4±1 hours after application)	Dislodging Solution
A1-09	01	Untreated	DAY 3-4, POST 1	3-4 days after application #1	Dislodging Solution
A1-10	02	Active Ingredient	DAY 3-4, POST 1	3-4 days after application #1	Dislodging Solution
A1-11	02	Active Ingredient	DAY 3-4, POST 1	3-4 days after application #1	Dislodging Solution
A1-12	02	Active Ingredient	DAY 3-4, POST 1	3-4 days after application #1	Dislodging Solution

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Table 2: Samples for Dislodgeable Foliar Residue Determinations (continued)

SAMPLE ID	TRT #	TRT	SAMPLING INTERVAL[1]	TIMING	SAMPLE MATRIX
A2-13	01	Untreated	PRE 2	1 day prior or on the day of (but prior to) application #2	Dislodging Solution
A2-14	02	Active Ingredient	PRE 2	1 day prior or on the day of (but prior to) application #2	Dislodging Solution
A2-15	02	Active Ingredient	PRE 2	1 day prior or on the day of (but prior to) application #2	Dislodging Solution
A2-16	02	Active Ingredient	PRE 2	1 day prior or on the day of (but prior to) application #2	Dislodging Solution
A2-17	01	Untreated	POST 2	day of application #2	Dislodging Solution
A2-18	02	Active Ingredient	POST 2	day of application #2 (4±1 hours after application)	Dislodging Solution
A2-19	02	Active Ingredient	POST 2	day of application #2 (4±1 hours after application)	Dislodging Solution
A2-20	02	Active Ingredient	POST 2	day of application #2 (4±1 hours after application)	Dislodging Solution
A3-21	01	Untreated	PRE 3	1 day prior or on the day of (but prior to) application #3	Dislodging Solution
A3-22	02	Active Ingredient	PRE 3	1 day prior or on the day of (but prior to) application #3	Dislodging Solution
A3-23	02	Active Ingredient	PRE 3	1 day prior or on the day of (but prior to) application #3	Dislodging Solution
A3-24	02	Active Ingredient	PRE 3	1 day prior or on the day of (but prior to) application #3	Dislodging Solution
A3-25	01	Untreated	POST 3	day of application #3	Dislodging Solution
A3-26	02	Active Ingredient	POST 3	day of application #3 (4±1 hours after application)	Dislodging Solution
A3-27	02	Active Ingredient	POST 3	day of application #3 (4±1 hours after application)	Dislodging Solution
A3-28	02	Active Ingredient	POST 3	day of application #3 (4±1 hours after application)	Dislodging Solution
A4-29	01	Untreated	PRE 4	1 day prior or on the day of (but prior to) application #4	Dislodging Solution
A4-30	02	Active Ingredient	PRE 4	1 day prior or on the day of (but prior to) application #4	Dislodging Solution
A4-31	02	Active Ingredient	PRE 4	1 day prior or on the day of (but prior to) application #4	Dislodging Solution
A4-32	02	Active Ingredient	PRE 4	1 day prior or on the day of (but prior to) application #4	Dislodging Solution

AAFC DISLODGEABLE FOLIAR RESIDUE STUDY PLAN
***Active Ingredient*:**
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES
STUDY #: AAFCXX-XXXD

Table 2: Samples for Dislodgeable Foliar Residue Determinations (continued)

SAMPLE ID	TRT #	TRT	SAMPLING INTERVAL[1]	TIMING	SAMPLE MATRIX
A4-33	01	Untreated	POST 4	day of application #4	Dislodging Solution
A4-34	02	Active Ingredient	POST 4	day of application #4 (4±1 hours after application)	Dislodging Solution
A4-35	02	Active Ingredient	POST 4	day of application #4 (4±1 hours after application)	Dislodging Solution
A4-36	02	Active Ingredient	POST 4	day of application #4 (4±1 hours after application)	Dislodging Solution
A4-37	01	Untreated	DAY 1, POST 4	1 day after application #4	Dislodging Solution
A4-38	02	Active Ingredient	DAY 1, POST 4	1 day after application #4	Dislodging Solution
A4-39	02	Active Ingredient	DAY 1, POST 4	1 day after application #4	Dislodging Solution
A4-40	02	Active Ingredient	DAY 1, POST 4	1 day after application #4	Dislodging Solution
A4-41	01	Untreated	DAY 2, POST 4	2 days after application #4	Dislodging Solution
A4-42	02	Active Ingredient	DAY 2, POST 4	2 days after application #4	Dislodging Solution
A4-43	02	Active Ingredient	DAY 2, POST 4	2 days after application #4	Dislodging Solution
A4-44	02	Active Ingredient	DAY 2, POST 4	2 days after application #4	Dislodging Solution
A4-45	01	Untreated	DAY 4, POST 4	4 days after application #4	Dislodging Solution
A4-46	02	Active Ingredient	DAY 4, POST 4	4 days after application #4	Dislodging Solution
A4-47	02	Active Ingredient	DAY 4, POST 4	4 days after application #4	Dislodging Solution
A4-48	02	Active Ingredient	DAY 4, POST 4	4 days after application #4	Dislodging Solution
A4-49	01	Untreated	DAY 7, POST 4	7 days after application #4	Dislodging Solution
A4-50	02	Active Ingredient	DAY 7, POST 4	7 days after application #4	Dislodging Solution
A4-51	02	Active Ingredient	DAY 7, POST 4	7 days after application #4	Dislodging Solution

AAFC DISLODGEABLE FOLIAR RESIDUE STUDY PLAN
***Active Ingredient*:**
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES
STUDY #: AAFCXX-XXXD

Table 2: Samples for Dislodgeable Foliar Residue Determinations (continued)

SAMPLE ID	TRT #	TRT	SAMPLING INTERVAL[1]	TIMING	SAMPLE MATRIX
A4-52	02	Active Ingredient	DAY 7, POST 4	7 days after application #4	Dislodging Solution
A4-53	01	Untreated	DAY 10, POST 4	10 days after application #4	Dislodging Solution
A4-54	02	Active Ingredient	DAY 10, POST 4	10 days after application #4	Dislodging Solution
A4-55	02	Active Ingredient	DAY 10, POST 4	10 days after application #4	Dislodging Solution
A4-56	02	Active Ingredient	DAY 10, POST 4	10 days after application #4	Dislodging Solution
A4-57	01	Untreated	DAY 14, POST 4	14 days after application #4	Dislodging Solution
A4-58	02	Active Ingredient	DAY 14, POST 4	14 days after application #4	Dislodging Solution
A4-59	02	Active Ingredient	DAY 14, POST 4	14 days after application #4	Dislodging Solution
A4-60	02	Active Ingredient	DAY 14, POST 4	14 days after application #4	Dislodging Solution
A4-61	01	Untreated	DAY 21, POST 4	21 days after application #4	Dislodging Solution
A4-62	02	Active Ingredient	DAY 21, POST 4	21 days after application #4	Dislodging Solution
A4-63	02	Active Ingredient	DAY 21, POST 4	21 days after application #4	Dislodging Solution
A4-64	02	Active Ingredient	DAY 21, POST 3	21 days after application #4	Dislodging Solution
A4-65	01	Untreated	DAY 28, POST 4	28 days after application #4	Dislodging Solution
A4-66	02	Active Ingredient	DAY 28, POST 4	28 days after application #4	Dislodging Solution
A4-67	02	Active Ingredient	DAY 28, POST 4	28 days after application #4	Dislodging Solution
A4-68	02	Active Ingredient	DAY 28, POST 4	28 days after application #4	Dislodging Solution

Table 2: Samples for Dislodgeable Foliar Residue Determinations (continued)

SAMPLE ID	TRT #	TRT	SAMPLING INTERVAL[1]	TIMING	SAMPLE MATRIX
A4-69	01	Untreated	DAY 35, POST 4	35 days after application #4	Dislodging Solution
A4-70	02	Active Ingredient	DAY 35, POST 4	35 days after application #4	Dislodging Solution
A4-71	02	Active Ingredient	DAY 35, POST 4	35 days after application #4	Dislodging Solution
A4-72	02	Active Ingredient	DAY 35, POST 4	35 days after application #4	Dislodging Solution

[1] Timing of sample interval is also outlined in Section 18A.

Table 3: Samples for Field Recovery and Travel Recovery

SAMPLE ID	TRT #	SAMPLING INTERVAL	SAMPLE DESCRIPTION	TIMING	CONCENTRATION OF FORTIFICATION SOLUTION (*u*g/200mL solution)*
A1-05**	01	POST 1	CONTROL	day of application #1	NA
F-01	01	POST 1	Field Recovery	day of application #1	**LOW** *Active ingredient:*
F-02	01	POST 1	Field Recovery	day of application #1	**LOW** *Active ingredient:*
F-03	01	POST 1	Field Recovery	day of application #1	**LOW** *Active ingredient:*
F-04	01	POST 1	Field Recovery	day of application #1	**HIGH** *Active ingredient:*
F-05	01	POST 1	Field Recovery	day of application #1	**HIGH** *Active ingredient:*
F-06	01	POST 1	Field Recovery	day of application #1	**HIGH** *Active ingredient:*
TR-L1	NA	POST 1	Travel Recovery	day of application #1	**LOW** *Active ingredient:*
TR-H1	NA	POST 1	Travel Recovery	day of application #1	**HIGH** *Active ingredient:*
A2-17**	01	POST 2	CONTROL	day of application #2	NA
F-07	01	POST 2	Field Recovery	day of application #2	**LOW** *Active ingredient:*
F-08	01	POST 2	Field Recovery	day of application #2	**LOW** *Active ingredient:*

AAFC DISLODGEABLE FOLIAR RESIDUE STUDY PLAN
***Active Ingredient*:**
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES
STUDY #: AAFCXX-XXXD

Table 3: Samples for Field Recovery and Travel Recovery (continued)

SAMPLE ID	TRT #	SAMPLING INTERVAL	SAMPLE DESCRIPTION	TIMING	CONCENTRATION OF FORTIFICATION SOLUTION (*u*g/200mL solution)*
F-09	01	POST 2	Field Recovery	day of application #2	**LOW** *Active ingredient:*
F-10	01	POST 2	Field Recovery	day of application #2	**HIGH** *Active ingredient:*
F-11	01	POST 2	Field Recovery	day of application #2	**HIGH** *Active ingredient:*
F-12	01	POST 2	Field Recovery	day of application #2	**HIGH** *Active ingredient:*
TR-L2	NA	POST 2	Travel Recovery	day of application #2	**LOW** *Active ingredient:*
TR-H2	NA	POST 2	Travel Recovery	day of application #2	**HIGH** *Active ingredient:*
A3-25**	01	POST 3	CONTROL	day of application #3	NA
F-13	01	POST 3	Field Recovery	day of application #3	**LOW** *Active ingredient:*
F-14	01	POST 3	Field Recovery	day of application #3	**LOW** *Active ingredient:*
F-15	01	POST 3	Field Recovery	day of application #3	**LOW** *Active ingredient:*
F-16	01	POST 3	Field Recovery	day of application #3	**HIGH** *Active ingredient:*
F-17	01	POST 3	Field Recovery	day of application #3	**HIGH** *Active ingredient:*
F-18	01	POST 3	Field Recovery	day of application #3	**HIGH** *Active ingredient:*
TR-L3	NA	POST 3	Travel Recovery	day of application #3	**LOW** *Active ingredient:*
TR-H3	NA	POST 3	Travel Recovery	day of application #3	**HIGH** *Active ingredient:*
A4-33**	01	POST 4	CONTROL	day of application #4	NA
F-19	01	POST 4	Field Recovery	day of application #4	**LOW** *Active ingredient:*
F-20	01	POST 4	Field Recovery	day of application #4	**LOW** *Active ingredient:*
F-21	01	POST 4	Field Recovery	day of application #4	**LOW** *Active ingredient:*

AAFC DISLODGEABLE FOLIAR RESIDUE STUDY PLAN
***Active Ingredient*:**
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES
STUDY #: AAFCXX-XXXD

Table 3: Samples for Field Recovery and Travel Recovery (continued)

SAMPLE ID	TRT #	SAMPLING INTERVAL	SAMPLE DESCRIPTION	TIMING	CONCENTRATION OF FORTIFICATION SOLUTION (*u*g/200mL solution)*
F-22	01	POST 4	Field Recovery	day of application #4	**HIGH** *Active ingredient:*
F-23	01	POST 4	Field Recovery	day of application #4	**HIGH** *Active ingredient:*
F-24	01	POST 4	Field Recovery	day of application #4	**HIGH** *Active ingredient:*
TR-L4	NA	POST 4	Travel Recovery	day of application #4	**LOW** *Active ingredient:*
TR-H4	NA	POST 4	Travel Recovery	day of application #4	**HIGH** *Active ingredient:*
A4-49**	01	DAY 7, POST 4	CONTROL	7 days after application #4	NA
F-25	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**LOW** *Active ingredient:*
F-26	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**LOW** *Active ingredient:*
F-27	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**LOW** *Active ingredient:*
F-28	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**HIGH** *Active ingredient:*
F-29	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**HIGH** *Active ingredient:*
F-30	01	DAY 7, POST 4	Field Recovery	7 days after application #4	**HIGH** *Active ingredient:*
TR-L5	NA	DAY 7, POST 4	Travel Recovery	7 days after application #4	**LOW** *Active ingredient:*
TR-H5	NA	DAY 7, POST 4	Travel Recovery	7 days after application #4	**HIGH** *Active ingredient:*
A4-57**	01	DAY14, POST 4	CONTROL	14 days after application #4	**NA**
F-31	01	DAY14, POST 4	Field Recovery	14 days after application #4	**LOW** *Active ingredient:*
F-32	01	DAY14, POST 4	Field Recovery	14 days after application #4	**LOW** *Active ingredient:*
F-33	01	DAY14, POST 4	Field Recovery	14 days after application #4	**LOW** *Active ingredient:*
F-34	01	DAY14, POST 4	Field Recovery	14 days after application #4	**HIGH** *Active ingredient:*

AAFC DISLODGEABLE FOLIAR RESIDUE STUDY PLAN
***Active Ingredient*:**
DISLODGEABLE FOLIAR RESIDUE DISSIPATION FROM GREENHOUSE TOMATOES
STUDY #: AAFCXX-XXXD

Table 3: Samples for Field Recovery and Travel Recovery (continued)

SAMPLE ID	TRT #	SAMPLING INTERVAL	SAMPLE DESCRIPTION	TIMING	CONCENTRATION OF FORTIFICATION SOLUTION (*u*g/200mL solution)*
F-35	01	DAY14, POST 4	Field Recovery	14 days after application #4	**HIGH** *Active ingredient:*
F-36	01	DAY14, POST 4	Field Recovery	14 days after application #4	**HIGH** *Active ingredient:*
TR-L6	NA	DAY14, POST 4	Travel Recovery	14 days after application #4	**LOW** *Active ingredient:*
TR-H6	NA	DAY14, POST 4	Travel Recovery	14 days after application #4	**HIGH** *Active ingredient:*
A4-61**	01	DAY21, POST 4	CONTROL	21 days after application #4	**NA**
F-37	01	DAY21, POST 4	Field Recovery	21 days after application #4	**LOW** *Active ingredient:*
F-38	01	DAY21, POST 4	Field Recovery	21 days after application #4	**LOW** *Active ingredient:*
F-39	01	DAY21, POST 4	Field Recovery	21 days after application #4	**LOW** *Active ingredient:*
F-40	01	DAY21, POST 4	Field Recovery	21 days after application #4	**HIGH** *Active ingredient:*
F-41	01	DAY21, POST 4	Field Recovery	21 days after application #4	**HIGH** *Active ingredient:*
F-42	01	DAY21, POST 4	Field Recovery	21 days after application #4	**HIGH** *Active ingredient:*
TR-L7	NA	DAY21, POST 4	Travel Recovery	21 days after application #4	**LOW** *Active ingredient:*
TR-H7	NA	DAY21, POST 4	Travel Recovery	21 days after application #4	**HIGH** *Active ingredient:*
A4-65**	01	DAY28, POST 4	CONTROL	28 days after application #4	**NA**
F-43	01	DAY28, POST 4	Field Recovery	28 days after application #4	**LOW** *Active ingredient:*
F-44	01	DAY28, POST 4	Field Recovery	28 days after application #4	**LOW** *Active ingredient:*
F-45	01	DAY28, POST 4	Field Recovery	28 days after application #4	**LOW** *Active ingredient:*
F-46	01	DAY28, POST 4	Field Recovery	28 days after application #4	**HIGH** *Active ingredient:*

Table 3: Samples for Field Recovery and Travel Recovery (continued)

SAMPLE ID	TRT #	SAMPLING INTERVAL	SAMPLE DESCRIPTION	TIMING	CONCENTRATION OF FORTIFICATION SOLUTION (*u*g/200mL solution)*
F-47	01	DAY28, POST 4	Field Recovery	28 days after application #4	**HIGH** *Active ingredient:*
F-48	01	DAY28, POST 4	Field Recovery	28 days after application #4	**HIGH** *Active ingredient:*
TR-L8	NA	DAY28, POST 4	Travel Recovery	28 days after application #4	**LOW** *Active ingredient:*
TR-H8	NA	DAY28, POST 4	Travel Recovery	28 days after application #4	**HIGH** *Active ingredient:*
A4-69**	01	DAY35, POST 4	CONTROL	35 days after application #4	**NA**
F-49	01	DAY35, POST 4	Field Recovery	35 days after application #4	**LOW** *Active ingredient:*
F-50	01	DAY35, POST 4	Field Recovery	35 days after application #4	**LOW** *Active ingredient:*
F-51	01	DAY35, POST 4	Field Recovery	35 days after application #4	**LOW** *Active ingredient:*
F-52	01	DAY35, POST 4	Field Recovery	35 days after application #4	**HIGH** *Active ingredient:*
F-53	01	DAY35, POST 4	Field Recovery	35 days after application #4	**HIGH** *Active ingredient:*
F-54	01	DAY35, POST 4	Field Recovery	35 days after application #4	**HIGH** *Active ingredient:*
TR-L9	NA	DAY35, POST 4	Travel Recovery	35 days after application #4	**LOW** *Active ingredient:*
TR-H9	NA	DAY35, POST 4	Travel Recovery	35 days after application #4	**HIGH** *Active ingredient:*

***Solutions to be prepared by laboratory given in Section 26, according to the procedure in Section 27 at the stated concentration.**
****These control samples are the same untreated samples listed in Table 2 for the same sampling interval. They can also be run as the controls for the field recovery samples.**

22. SAMPLE STORAGE AND SHIPMENT:

After residue dislodging operation, place the samples into a freezer. Samples must be placed into a freezer as soon as possible or within 1 hour following the completion of dislodging operation. For pre-shipment storage, the samples will be held frozen at temperatures generally less than –18°C, allowing for normal variations due to freezer cycling, sample movement, etc. Time into and an inventory of the freezer will be documented. All max/min storage temperatures will be

monitored and documented. Sample containers will be placed at an angle until the solutions are frozen. Container lids will be wrapped with parafilm or other wrap to prevent sample evaporation and contamination after tightening. After the samples are frozen the sample containers will be packed in a manner to minimize contact with each other during shipment. The samples are to remain frozen while in transit to the analytical laboratory via freezer truck or "express" shipment. Shipments sent via express shipment (overnight carriers such as Federal Express or Purolator) will require the addition of quantities of dry ice sufficient to maintain sample integrity while in transit to the laboratory. A completed chain of custody form will accompany the samples during shipment along with an inventory outlining each sample within the shipment. Document the notification made to the sample destination by use of e-mail, fax, telephone log, raw data field notebook, communication note, etc. All packing and shipping procedures and notification made to the sample destination by use of e-mail, fax, or telephone log will be documented in the RDFN. **Send samples to the laboratory identified in the table below. This should be done as soon as practical, avoid shipments from Thursday through Sunday**

Trial ID No.	**Ship to: (Trial ID No., Contact and Shipping Address)**
AAFCXX-XXXD-XXX	AAFCXX-XXXD-XXX Attn: Phone: Fax: Email: See section 26 for responsible person for this trial ID No.

23. GREENHOUSE DOCUMENTATION AND RECORD KEEPING:

All operations, data and observations appropriate to this study should be recorded directly and **promptly** into the RDFN. The content of the RDFN should be sufficiently detailed to completely reconstruct the field trial. At a minimum, collect and maintain the following raw data:

- Names of all personnel conducting specific research functions
- Study plan amendments relevant to the greenhouse trial
- Deviations from study plan and standard operating procedures
- Trial site greenhouse information, including historic pesticide use
- Plot maps
- Test item receipt, and fortification solution receipt, use and disposition records
- Test item and fortification solution storage conditions (including minimum and maximum temperatures)
- Data regarding calibration and use of application equipment
- Treatment application
- Crop maintenance pesticides, crop production and cultural practices
- Residue sample, field recovery sample and travel recovery sample identification, collection, storage conditions and handling
- Procedures of sample dislodging and field recovery samples
- A record of sample handling, including time of collection to freezer and a copy of the freezer temperature logs for the period of time the samples were stored
- Sample shipping information

- Daily greenhouse records for the trial period (growing period). Data should include: environmental conditions, type of growth medium and its composition, irrigation, air exchange, light regime, and greenhouse pesticide maintenance records for all products applied over the growth period.
- Pass times (if applicable) and other data to confirm amount of material applied to plots
- Other applicable data requested in the RDFN that are needed to prove that the conduct of the study was in accordance with the study plan.

24. STUDY PLAN/SOP MODIFICATIONS - GREENHOUSE RESEARCH:

Consult with the Study Director regarding desired changes to the Study Plan prior to occurrence. If appropriate an amendment will be issued. Any deviations to the Study Plan or to a Standard Operating Procedure will require the Principal Investigator or Study Director to complete a deviation form. Any deviation should be communicated to the Study Director either verbally, by fax or email within **48 hours** (document in communication log) and in writing on the form provided, within **7 days** of occurrence or recognition. The Study Director will assess the impact of the deviation on the study and act accordingly.

25. RAW DATA FIELD NOTEBOOK /ARCHIVING:

The Principal Investigator will ensure that the completed **original** RDFN is forwarded to GLP Admin after sample shipment and appropriate review. The Principal Investigator will maintain a scan or printed copy of these field documents.

26. LABORATORY PERSONNEL/TRIAL ID NO.:

(Responsible for Sections 27-38)
The Principal Investigator and test site management must sign the GLP Acceptance form (Appendix A) and return as directed.

PRINCIPAL INVESTIGATOR: **TRIAL ID No.**

TEST SITE MANAGEMENT:

27. PREPARATION OF THE FORTICIFCATION SOLUTIONS FOR FIELD RECOVERY AND TRAVEL RECOVERY:

The analytical laboratory will prepare a series of fortification solutions at two concentrations, to be used for the field recovery samples at the field location and as travel recovery samples. A low concentration solution will be prepared with the reference item (in methanol) to contain X µg/mL of *active ingredient* per 1.0 mL of the fortification solution. A high concentration solution (in methanol) will contain X µg/mL of *active ingredient* per 1.0 mL of the fortification solution. From the low concentration solution, 1.0 mL will be dispensed into separate amber-vials with Teflon-lined screw tops as required; mark the vials containing with an “L”, using an indelible marker (e.g., Sharpie). Similarly, for the high concentration solution, 1.0 mL will be dispensed into separate amber-vials with Teflon-lined screw tops as required; mark the vials containing with an “H”. The volume of solution in each will be indicated by a horizontal line marked on the vial. The sample vials will be chosen such that the in-field fortification samples can be fortified as specified above in

Section 20, and are to be clearly identified as to contents. Plus 6 additional contingency vials, for a total of at least **42** vials per concentration.

The analytical laboratory will ship fortification solutions in two batches. The first shipment will provide enough fortification solution vials for the test site to complete fortifications up to seven days after application #4. The second shipment will provide enough fortification solution vials to allow the test site to continue fortification up to 35 days after application #4. Fortification solutions will be shipped to the greenhouse test site via overnight courier with sufficient ice to remain cool. The analytical laboratory is to include, with each shipment instructions for fortification and instructions to store the fortification solutions.

28. LABORATORY SAMPLE INVENTORY:

Untreated and treated dislodgeable foliar residue samples, fortification samples, fortification solution samples and travel controls will be received from the field site(s) outlined in Section 22 (for responsible persons see Section 10). Notify the appropriate Principal Investigator and Study Director of sample receipt by returning (by fax, email, or mail) a copy of the completed Chain of Custody form or a similar laboratory form used for sample arrival confirmation.

29. LABORATORY SAMPLE IDENTIFICATION:

Each sample (as received from the test site - treated, controls, method validation, field recovery, travel recovery, etc.) is to be assigned a unique laboratory sample number by the laboratory personnel (Note, use of the field sample identification number is acceptable - Refer to Tables 1, 2 and 3). A cross-reference must be maintained between the assigned laboratory sample number and the identification utilized in the Sample Chain of Custody Form received from the field sites. Both identification numbers must be reported in the analytical report.

30. LABORATORY SAMPLE STORAGE/PREPARATION:

Store samples in a limited access area at temperatures that will maintain frozen sample integrity [generally less than –18°C (0°F)], allowing for normal variations due to freezer cycling, sample movement, etc] until extraction. All storage temperatures, conditions and location of sample storage must be monitored and documented.

31. LABORATORY REFERENCE ITEM:

The laboratory will use reference item(s), *active ingredient,* obtained from the Registrant. Contact *name*, *company*, Tel:, Fax:, e-mail: to procure the reference item(s). Document the date the reference item(s) is received, the source, lot number, stated purity, storage conditions, and expiration date. Use only reference item(s) that have been characterized to meet GLP standards. Characterization of the reference item(s) (purity, identity, stability, and solubility) and maintenance of an archival sample is the responsibility of the registrant unless otherwise specified by the Study Director.

32. STANDARD SOLUTION PREPARATION AND STABILITY:

At a minimum, run five calibration standards, prepared from at least two different stock solutions (i.e., individually prepared from different weighings of the reference item). Use at least two calibration standard solutions per stock solution from different weighings to develop the calibration curve and ensure that the concentration range includes the LOQ towards the lower end of the

curve. It is also imperative that the LOQ is not the lowest or highest standard used. The analyte response area/height for the calibration standards must bracket the analyte response area/height of the fortification samples and treated samples with residues above the LOQ. The use of a zero standard/blank as part of the calibration series is not acceptable.

If standard and stock solutions are not prepared fresh daily and unless stability over the time period for which the solution has been prepared has been previously demonstrated (in the same solvent and storage conditions), their stability needs to be verified. This is to be done by analysing a solvent/reagent blank, to ensure there is no interference, and then comparing the response factor (typically five replicate injections) of the aged stock solution (aged for the longest time period the standard used during sample analysis has been stored for) to the response factor (typically five replicate injections) of a freshly prepared stock solution. The analyte will be considered stable in solution if the response factor of the aged standard is within ±10% of the freshly prepared standards. Values outside of this range may require re-analysis as determined by the Study Director and Principal Investigator. Data is available indicating that standard solutions for Active Ingredient are stable for up to X days.

33. ANALYTICAL METHODOLOGY:

REFERENCE METHOD(S):
There is not a validated DFR method for Active Ingredient. The plant residue methods described below are acceptable for use in this DFR study. **Any method modifications must be done in consultation with the Study Director** and receive Study Director approval before making any changes.

Enter method name

In each analytical set, the calibration standards must be injected before the first and after last field-treated sample. Additional calibration standards should be interspersed between samples to ensure goodness of fit to the standard curve.

All field and fortified sample injections should be made in duplicate. The difference in response should be less than ±10%. Otherwise, the vial should be re-injected in duplicate or other remedial action taken. The mean residue value from the two injections is to be reported and used in all subsequent calculations.

If matrix-matched standards are used to develop the validation calibration curve, then a calibration curve using solvent-based standards must also be generated for comparison. Study Director approval is required to continue use of matrix-matched standards.
If modifications to the reference method results in a lower achievable LOD and LOQ, the laboratory may have to establish a new LOD (based on S:N criterion) for the working method and validate the method at the new LOQ. The Study Director should be consulted if this situation arises.

REFERENCE METHOD MODIFICATIONS/METHOD VALIDATION:
It may be necessary to modify the reference method, if interferences arise due to the test matrix. However, the reference method, along with any modifications must be validated individually for each compound in the Residue Definition [ ], prior to residue sample analysis of the test matrix. **Document the exact working method, with "stopping points", that will be used for sample**

analysis. This validated step-by-step working method must incorporate and outline all changes from the reference method. Provide the Study Director with a copy of this working method, along with the results of method validation (including chromatograms of the standards, fortified samples and control (untreated) sample), **for Study Director approval, prior to analyzing the treated samples**.

The method must be validated using the **untreated samples** outlined in Section 21 **(Table 1)** prior to the receipt of the treated greenhouse samples. The field PI will supply the laboratory PI with samples of dislodging solution from untreated leaf disks that were processed similar to the treated disks for method validation. To validate the method, analyse a minimum of one control sample and a minimum of three replicates, at each of four levels of fortification: 1) LOQ: X *u*g *active ingredient* in 200 mL of dislodging solution, 2) 10 x LOQ: X *u*g *active ingredient* in 200 mL of dislodging solution, 3) 100 x LOQ: XX *u*g *active ingredient* in 200 mL of dislodging solution and 4) 500 x LOQ: XXX *u*g *active ingredient* in 200 mL of dislodging solution. The control sample is to be analysed either immediately following the highest analytical standard or after the XXX *u*g/XXX *u*g per sample extract to demonstrate that sample carryover is not occurring. The acceptable recovery range is 70-120%, with an overall %RSD of ≤ 20%. Documented approval from the Study Director is needed for all recoveries outside of this range. During sample analysis, if residue levels are greater than XXX *u*g *active ingredient* per sample, the method validation will be extended by analyzing three replicates of a control sample fortified at or within 10 times the highest level of residues found. An additional control sample is to be analysed after the fortified samples.

SAMPLE ANALYSIS:
Samples will be analyzed and reported for the Residue Definition of the test item, i.e., [*active ingredient*], following the successful validation of the working method. Analyze at least one control and all treated residue samples for each matrix, in the same manner as that used for the method validation. Contact the Study Director immediately if residues above 20% of the lowest level of method validation for the matrix are detected in the control samples. **All changes or modifications to the working method require Study Director approval**. Whenever possible, notify the Study Director prior to occurrence. Any change or modification to the working method must be documented in the raw data and discussed in the final analytical report.

In addition to the treated samples, at least one concurrent fortification sample for each compound in the residue definition being determined and one control sample will be extracted per analytical set. The Study Director must be notified immediately if concurrent recoveries deviate from the acceptable recovery range of 70% to 120%. All efforts will be made to resolve existing recovery problems before continuing forward with additional analytical sets. Samples with area counts/heights that exceed the calibrated range will be diluted accordingly, and re-injected in a timely manner. A minimum of one concurrent fortification sample per analytical set at the limit of quantification (LOQ) is required. Six concurrent fortification samples at the LOQ must be analyzed during the sample analysis phase. Over the course of residue sample analysis, adequate concurrent recovery samples that **bracket the actual residues** must be analyzed. A solvent/reagent blank will be injected with each set analyzed during the course of this study.

Generally, sample extracts should be stored in a refrigerator or freezer, for no longer than 14 days before injection. Contact Study Director if samples extracts are stored greater than 14 days prior to injection.

Beyond application #4 if residues for two consecutive intervals are non-detect (≤ LOQ) sample analysis will no longer be necessary.

Sample Sets: Each analytical sample set is to include, but not be limited to, experimental samples (both untreated/control and treated), two concurrent fortifications at levels of fortification identified below, a reagent/solvent blank and calibration standards as appropriate for the analytical method. Throughout the course of sample analysis, a minimum of one reagent blank and a field recovery sample (for each concentration) must be analyzed. The **untreated samples** provided by the field site from **Table 2** should be used for the control, and concurrent fortification samples.

Fortification Level(s): Two concurrent fortification samples are to be included with each sample set. Method fortifications are to include levels of approximately 0.5/1, 5/10, 50/100, and 250/500 *µ*g *active ingredient* in 200 mL dislodging solution during sample analysis. Actual levels of fortification will be documented in the raw data. These levels are intended to bracket the range of the expected residues in the treated samples. If residues exceed the above range, the Study Director must be contacted.

Travel Recoveries: To verify the integrity of the in-field fortification solutions, the contents of travel recovery vials returned from the field site are to be quantitatively added to 200 mL of dislodging solution and analyzed. Three replicates are required.
Note: If field recovery samples are within acceptable limits, the travel recoveries do not need to be analyzed.

Limit of Quantification: Proposed to be X *u*g *active ingredient* in a 200 mL dislodging solution sample.

Interference or Detection in Control Samples: Responses detected above ½ the LOQ in any control samples will be brought to the attention of the Study Director (within 24 hours of occurrence).

Storage Stability Analysis:
As soon as possible after receipt of the samples, sub-samples of the dislodging solution from control samples (a minimum of 3 per time frame), are to be fortified at the equivalent of XX ug *active ingredient* per 200 mL and put in frozen storage; a smaller volume of dislodging solution (e.g., 20 mL) can be used for the storage sample. A 20 day stability analysis schedule will be followed extending to 60 days. An additional analysis date will be carried out if the longest sample storage interval falls in between 60 and 80 days. Three samples of each analyte will be analyzed after the appropriate storage period beginning at 0-day then 20±1, 40±1, 60±1 days thereafter and longer if necessary. As well, one unfortified and three freshly fortified controls will also be included at each time frame.

Statistical Method(s): Linear or non-linear regression (weighted or non-weighted) may be used to generate the standard curves. The same type of curve must be used throughout the study. Curves will have an r^2 that is ≥ 0.99. Means and standard deviations are to be reported for method validation samples and procedural (concurrent) recoveries.

34. DISPOSITION OF SAMPLES:

Sample extracts can be disposed of after data analysis.

35. LABORATORY STUDY PLAN/SOP MODIFICATIONS - LABORATORY RESEARCH:

Consult with the Study Director regarding desired changes to the Study Plan **prior to occurrence**. If appropriate, an amendment will be issued. Any unauthorized changes to the study plan will require the Principal Investigator to complete a deviation outlining the changes. This deviation should be provided to the Study Director promptly (e.g., within 24 hours of noticing the occurrence) for review and signature. All deviations from the approved SOP's also require documentation and approval by the Study Director.

36. LABORATORY DOCUMENTATION AND RECORD KEEPING:

A study file shall be developed and maintained by the Principal Investigator in conjunction with the analysis. It will contain a true copy of the study plan, all pertinent raw data, documentation, records, correspondence, and the final analytical report. In addition, records of equipment maintenance and calibrations will be maintained and archived by the laboratory facility.
All operations, data, and observations shall be recorded in the analyst's notebook, log books, or forms, which must be signed and dated upon entry. All pages of the raw data should include the trial ID#. At a minimum, collect and maintain the following raw data:

- Names of personnel conducting specific research functions
- Chain of custody records
- Reference item(s), Certificate of Analysis, receipt, use, storage conditions and disposition records
- Sample storage conditions and locations
- Standard solution(s) and prepared reagents: storage conditions, dilution calculations and preparation records
- Solvent(s) name, lot number, expiration date and source (manufacturer)
- Sample analysis worksheets
- Concurrent recovery fortification records
- Storage stability fortification records
- All chromatograms, including those that are not reported
- Calculation work sheets, statistical assessment, (means, standard deviations)
- Deviations from study plan, working method and standard operating procedures

37. LABORATORY RESEARCH REPORT:

The final analytical report sent to the Study Director shall contain, but not be limited to:

- reference item identity including name, structure, purity, lot number, expiration date and source (manufacturer)
- cross-reference of sample identification numbers
- calibration standard weights and preparation procedures
- complete copy of the step by step analytical working method
- any modifications or deviations from the study plan and/or working method
- method validation data (if applicable)
- residue levels for untreated and treated samples with concurrent fortified recoveries
- summary of quantitative data associated with samples plus field and travel recovery samples should be provided (e.g., sample weights/volume, final volumes, injection volumes, peak areas/heights)
- storage stability data (if required in section 30)

Representative chromatograms including the following (Note a "set" represents an analytical injection run done on a particular day):

- Calibration standards (for each analyte): A standard run, including one chromatogram for each concentration level used and the corresponding calibration curve. In addition, include one standard chromatogram from each set.
- Method validation (for each analyte): one chromatogram for each fortification level used (including method validation extensions).
- Concurrent recoveries (for each analyte): Chromatograms showing recoveries at the LOQ as well as the high level fortification.
- Controls (for each analyte): At least one untreated control (UTC) chromatogram per trial, ensuring these include one UTC chromatogram per set, as well as one UTC chromatogram that was run after a high level fortification sample.
- Treated samples (for each analyte): minimum of ten chromatograms (all if less than 10 in study), depicting representative samples per trial ID covering each day of harvest and covering each day of analysis (ensuring a mixture of the triplicate samples).
- Blanks: one reagent/solvent blank.
- Any chromatograms with unusual or inconsistent results.

- summary data associated with calibration standards and calibration curves
- clearly presented example calculations and statistical evaluations
- discussion of results (including purpose of method modifications, sample storage conditions)

38. LABORATORY ARCHIVES:

When the final analytical report is completed, the report and all original raw data will be sent to the Study Director. A "true copy" of the original raw data including the final analytical report shall be secured in the archives of the Principal Investigator/Testing Laboratory. The original raw data will be secured in the archives of the Sponsor once the study is completed.

APPENDIX A

GLP Acceptance Form

Trial ID #: AAFCXX-XXXD-_____

I acknowledge that I have read, and understood, the material contained in the assigned sections of this Study Plan. The research will be conducted in accordance with this Study Plan and the OECD GLP Principle of Good Laboratory Practices (revision 1997). Any work conducted in the USA will be conducted according to EPA Good Laboratory Practice standards, 40 CFR part 160, amended as effective Oct 16, 1989, which are acceptable to OECD standards. In addition, I will cooperate with the Quality Assurance Personnel in scheduling needed inspections and documenting responses to QA audit reports.

Principal Investigator

Printed Name	Signature	Date

Acknowledged by Test Site Manager:

Printed Name	Signature	Date

The following Individual or Company will be responsible for the Quality Assurance for this trial

Name of Quality Assurance *(Print)*

Form Completion and Return Instructions: At a minimum, the PI is to sign this form prior to performing any experimental work. Once the form has been completed, ***a copy of the form*** *needs to be inserted in the RDFN and the original must be returned to the individual identified below.*

GLP Admin
AAFC Minor Use Pesticide Program
Building 57, Central Experimental Farm
960 Carling Avenue
Ottawa, ON, Canada
K1A 0C6