1. PROJECT TITLE: ETHEPHON: Magnitude of the Residue on ORANGE

2. JUSTIFICATION AND OBJECTIVES:

IR-4 has received a request for the minor use of ethephon on orange to obtain better peel color on fresh market varieties.

To establish this tolerance, it is required that the magnitude of the residue in or on the commodity be determined as per EPA Series 860 Guidelines. The purpose of this study is to collect and analyze treated and untreated residue samples from appropriate field sites according to the application parameters requested to provide the sponsor with residue chemistry data to support a regional pesticide tolerance.

3. SPONSOR/TESTING FACILITY NAME, ADDRESS AND PHONE:

IR-4 Project Headquarters, 500 College Road East, Suite 201 W, Princeton, NJ 08540, (732) 932-9575, FAX# (609) 514-2612.

4. STUDY DIRECTOR[1]:

Raymond Leonard IR-4 Project Headquarters, 500 College Road East, Suite 201 W, Princeton, NJ 08540, (732) 932-9575 X4624, FAX# (609) 514-2612, e-mail: leonard@njaes.rutgers.edu

5. PROPOSED DATES:

Experimental Start :	03/20
Experimental Termination:	11/22
Study Completion:	10/23

6. STUDY DIRECTOR INITIALS: RCL

7. STUDY AUTHORIZATION:

Deborah Carpenter March 12, 2020	Raymond C Leonard March 14 2020
Sponsor Representative / Date	Raymond Leonard/ Study Director / Date

8. GOOD LABORATORY PRACTICE COMPLIANCE:

To determine the magnitude of residues of total ethephon in or on orange, this protocol will be employed using appropriate Standard Operating Procedures (SOP's) and will be conducted under provisions outlined in 40 CFR Part 160, in accordance with EPA's Good Laboratory Practice Standards. Canadian field/processing/analytical trials, if any, will be conducted at facilities consistent with the provisions outlined in the Organization for Economic Cooperation and Development (OECD) Series on Principles of Good Laboratory Practice and Compliance Monitoring.

The appropriate cooperative testing facility (field and laboratory) will be responsible for certifying that its portion of the study will be conducted in accordance with EPA's Good Laboratory Practice (GLP) Standards, 40 CFR 160, amended and effective Oct. 16, 1989. A statement of compliance, together with any GLP deviations will be signed and submitted by the appropriate Research Directors in their report or data package.

[1]In case the Study Director is not available, contact Dr. Deborah Carpenter (x4637) or Dr. Daniel Kunkel (x4616) at IR-4 Headquarters (732) 932-9575 for guidance.

9. QUALITY ASSURANCE:

Quality Assurance duties and responsibilities will be in conformance with 40 CFR 160.35. A Quality Assurance Statement will be submitted in the final report and shall include the date inspections were made and date(s) the findings were reported to the Study Director and management.

10. TEST SYSTEM/CROP:

ORANGE - Use a commercial variety. Report: variety, age of trees, and other descriptive information if available.

Field trials will be conducted at the appropriate sites to support the establishment/maintenance of a regional residue tolerance; see Section 23 **for these assignments**. Refer to Section 11.4 for requirements to differentiate multiple trials by the same field researcher.

11. TEST SYSTEM DESIGN and STATISTICAL METHOD:

11.1 Each test site will consist of one untreated and one treated plot **OR** two treated plots.

The individual plots shall be of adequate size to ensure that no more than 50% of the harvestable crop in the sampled area will be needed to provide the necessary plant material. See Parts 17 & 18 for requirements for residue sampling. The sampled crop must be commercially mature to be considered "harvestable", unless otherwise indicated in Part 15 or Part 17.

Field trial FL146 will provide samples for processing. The plots must be large enough to provide enough sample weight to meet processing requirements.

Field trial FL145 will provide samples for a decline trial (multiple sampling dates for the treated plot). The treated plot(s) must be large enough to provide enough samples on each sampling date to meet sample size requirements.

11.2 Employ adequate buffer zones between each of the plots to prevent contamination. For most application types, a minimum distance of 15 feet is required, but a minimum of 50 feet is strongly preferred. For applications made by airblast, mist blower, or power sprayers, a minimum distance of 50 feet is required, but a minimum of 100 feet is strongly preferred. When plants are used as a buffer between the untreated and treated plots, a lower distance is needed to prevent contamination, but the minimums indicated above must be observed. If another study using a test substance with the same active ingredient is being conducted at the same research site, the untreated plot from one study must be separated from the treated plot(s) of the other by the appropriate buffer zone indicated above.

11.3 If this pesticide use is not registered on this crop, federal law requires that the treated crop must be destroyed or handled in such a way that it is not consumed as a human food or animal feed.

11.4 This section applies when a Field Research Director (FRD) has been assigned more than one trial in this study, or when two or more trials assigned to different FRDs are located within 20 miles (32 km) of each other.
An independently prepared tank-mix must be used in each trial.
Also, choose at least one option from Set 1 **or** at least two options from Set 2:

Set	Option	Description
1	A	Trial sites must be separated by at least 20 miles (32 km) [measured as straight line distance]
	B	First application or planting date (for annual crops) in each trial is separated by at least 30 days
	C	Different crop variety (different size or shape at maturity, rough vs. smooth surface, different amount of foliage shielding the commodity, different rate of growth, or representative of the major varieties grown within the region)—confirm with Study Director if this option will be chosen
2	A	Spray volume must vary by at least 25% of the lower volume (minimum 10 GPA difference) Example 1, Trial A has a volume of 20 GPA and Trial B has a volume ≥ 30 GPA Example 2, Trial A has a volume of 60 GPA and Trial B has a volume ≥ 75 GPA The trial with the lowest spray volume for the first application must remain the lowest for each application; the trial with the highest must remain the highest for each, and so on
	B	NA
	C	NA
	D	NA
	E	Different types of application equipment be used in each trial (for example, tractor-pulled boom sprayer, tractor-pulled spreader, airblast sprayer, axial fan orchard sprayer, proptec sprayer, cannon mist sprayer, tower sprayer, over-row sprayer, tunnel sprayer, backpack sprayer, waist pack sprayer, hand gun, hand-held spreader, or shaker can)
	F	Different spray droplet size (fine, medium, coarse, very coarse, or extra coarse) This may be accomplished by changing nozzles and/or by changing spray pressure Document in the Field Data Book the droplet size that results from the pressure and nozzles used in the trial (nozzle catalog may be used as a reference) Coarse, very coarse, and extra coarse are appropriate for herbicides only
	G	NA
	H	NA
	I	Different irrigation type (drip or furrow or sprinkler/over-the-top) (Irrigation must be applied at least once after each application, but over-the-top irrigation must not be applied within one hour of an application, and irrigation is not needed following the last application if samples are to be collected on the same day)
	J	NA
	K	NA
	L	NA
	M	NA
	N	NA
	O	NA
	P	NA

If these criteria cannot be met to separate multiple trials, the Field Research Director should contact the Study Director to discuss possible alternatives that can be amended to the protocol. Trials conducted in different calendar years are exempt from these requirements.

11.5 Mark plots with identifiable markers containing at minimum the Field ID number and treatment number or treatment name that will persist for the duration of the field research trial or that can be readily replaced.

11.6 This study is not designed for statistical evaluation of field data.

12. TEST SITE PREPARATION:

Select a test site that has been maintained following good local agricultural practices for the production of oranges including fertilization, irrigation, if necessary and available, and other practices that ensure commercially acceptable crop production.

The test site will have a known pesticide and crop treatment history of a minimum of 1 year and preferably 3 years.

13. TEST/CONTROL SUBSTANCE:

IR-4 Headquarters personnel will arrange procurement of GLP test substance from the registrant. The registrant will provide a copy of the Certificate of Analysis to IR-4 Headquarters.

Use the Ethephon 2 Plant Regulator formulation (2 lb ai/gal) of ethephon (EPA Reg No. 66330-262, CAS# 16672-87-0) that has been characterized to meet GLP standards. IR-4 Headquarters personnel will arrange procurement of GLP test substance from the Registrant. Upon receipt, document the lot/batch number, condition, quantity received and if GLP characterized. **Temperature monitoring should begin within 2 days of receipt of the test substance, regardless of where it is held or stored.**

Contact the Study Director if there are any concerns regarding the GLP status, labeled identification, expiration date, etc. of the test substance.

EPA regulations require that test substance container(s) must be retained until the final study report is completed.

Study completion can be confirmed by contacting the Study Director or the Regional Field Coordinator, or by searching the IR-4 web site; click on "Food Crops" and under the "IR-4 Food Crops Database" click on the "Test Substance Container Disposal Approval" link. URL: http://ir4app.rutgers.edu/Ir4FoodPub/SubstanceDispoSch.aspx

Alternatively, some registrants will archive the test substance containers. If test substance containers are shipped to another location, the shipment must be conducted in accordance with local, state, and Federal regulations. See shipping documents for directions for return of the test substance; if none are given, contact the registrant representative: Sherry Hutcheson, UPL NA Inc., Phone: 229-247-9041, email: sherry.hutcheson@upl-ltd.com

Before the completion of this study, the Study Director shall receive confirmation from the registrant of the location of a retention sample of the test substance. Control substances are not relevant to this study.

An optional tracking form that may be used to confirm that the correct test substance has been received (with the correct label and Certificate of Analysis) is available at: http://wrir4.ucdavis.edu/Resources/Tricks/default.html .

14. TEST SUBSTANCE APPLICATION:

14.1 Simulate commercial application practices by applying the test substance in a manner representative of an application technique that is used by area commercial growers, at the application rate and timing specified in Section 15.

- Use application equipment that will provide uniform application of the test substance and result in adequate canopy penetration and coverage.
- The test substance, if applied in a mixture, must be applied to the test system within 30 minutes of mixing, otherwise the mixture must be agitated just prior to making the application to ensure that it is well mixed. (The additional agitation should be documented in Part 6G of the Field Data Book.) The mixture must always be applied to the test system within 2 hours of mixing.

- Each field trial requires a unique spray mixture. Do not use the spray mixture from one field trial on another field trial.

For foliar directed applications (generally used for insecticides and fungicides), do not proportionally reduce the application rate (the amount of active ingredient applied per acre). Direct the entire per-acre rate onto the crop. If row widths in the research plots are greater than local commercial practices, then the application rate should be calculated using a local commercial row width. **Note that the treated area for directed applications is calculated as row spacing X number of rows X plot length.** **Contact the Study Director** if guidance is needed.

All study participants are **reminded** and **encouraged** to follow all appropriate campus, local, state (or provincial) and national regulations and laws in association with the safe use of pesticides.

14.2 Full Calibrations for output and speed must be performed to ensure accurate delivery.
A calibration consists of a minimum of 3 consecutive, documented checks for nozzle or hopper output and speed (equipment or walking speed). An output calibration is a 3 run discharge of all the nozzles. An output recheck is a single run discharge of all the nozzles. A speed calibration is 3 runs. A speed recheck is a single run. (When the output of an airblast sprayer is calibrated or rechecked, it is not necessary to record the outputs of individual nozzles.)

Verification of the actual amount of test substance applied will always be made using **the most recent complete calibration data for that equipment.** (Note: When the most recent calibration data is from another trial, a certified true copy of that data must be included in the field data book for this trial.)

Discharge/Output Calibrations:

Is this the first application of test substance in this trial?

YES: A full calibration is required just prior to the first application (allowable the day before the application, but calibration on the day of use is preferred). A single, full calibration may be used for multiple trials in the same study or multiple studies if the following conditions are met:
1. The first application in each trial is the day of the calibration or the following day.
2. Application parameters and equipment components remain the same for each of the trials.
3. A recheck is run in each of the trials after the first.

NO: A single run recheck may be conducted to confirm consistent delivery (within ±5% of the last complete calibration) just prior to subsequent applications. (Full calibrations are preferred.)

Recheck is required when:
1. The equipment has been moved from the location where the most recent full calibration or recheck has occurred. (A sprayer that has been calibrated or rechecked at a farm or research station and then used to make an application somewhere else on that same farm or research station is *not* considered to have been "moved".)
2. The equipment has been cleaned.
3. Nozzles are removed and placed back on.
4. CO_2 tank has been changed.

Recheck is not required when the same Field Research Director is making applications on the same day for multiple trials in this study or separate studies, or multiple treatments in the same trial, unless there have been changes in other application parameters as described above.

Full output calibration is required if:
1. This is the first application in this trial

2. Application parameters or equipment components have changed (other than changing out CO_2 tanks) including:
    a. Nozzle or hopper output
    b. Nozzle size or type (full output calibration is not required if the same, clearly identified nozzles used for the full calibration have been placed back in the same positions on the boom after other nozzles have been used for another trial; in this case, only a recheck is needed)
    c. Change in delivery pressure by more than 5% (even if it has been changed back to the pressure used during the initial calibration UNLESS the pressure change is accomplished by replacing the regulator, and the screw on the regulator used in this trial has not been turned since the full calibration)
3. A recheck is not within ±5% of the last complete calibration.
4. The discharge of any single nozzle during a run of a full calibration or a recheck is greater than ±5% of the mean of the same run (this does not apply to airblast sprayers). If this occurs the nozzle must be adjusted or replaced, and a full calibration must be conducted to ensure that the nozzle discharge is within 5% of the mean and to determine a new output.

Target outputs: The use of a target output rather than the mean output may be used in the calculations made prior to the application; however, a "target check" calibration consisting of three runs must be conducted just prior to each use of a target output, and the mean output must be within 5% of the target output. Using a target output rather than a mean output increases the probability that an application rate deviation will occur. Verification of the amount of test substance that has been applied in calculations that use the discharge rate will always be made using the most recent calibration data.

Speed Calibrations:

Conduct the speed calibration in an area adjacent to the test plot, or on similar terrain (allowed the day before the application, but calibration on the day of use is preferred).

Is this the first application of test substance in this trial?

YES: A full speed calibration is required.
Exceptions: 1) When a handgun is used to spray tree fruits or nuts, and each tree is sprayed for a predetermined time, a speed calibration is not required and 2) When applications are made in multiple trials on the same site, same day, using the same equipment and same speed, a speed calibration is only required for the first application made that day.

NO: A single run recheck may be conducted to confirm consistent speed (±5% of the last complete speed calibration) just prior to subsequent applications.

Full speed calibration is required when:

1. A major equipment change has been made, such as from a tractor-pulled sprayer to a backpack sprayer.
2. A complete output calibration is performed.

Speed recheck is required when:

1. Speed calibration data from another trial is used, except for applications that are made on the same day on the same farm, using the same equipment and same speed.
2. Whenever an output recheck is performed, except for multiple applications within a study that are made on the same day on the same farm.

Speed recheck is not required when the same Field Research Director is making applications on the same day for multiple trials in this study, or multiple treatments in the same trial, unless there is a major equipment change or the treated plots are located on separate farms.

14.3 Actual Application Rate: Record actual application pass-times in the Field Data Book and verify the accuracy of the application against the protocol rate. **The application is considered acceptable if the accuracy is within -5% and +10% of the target rate specified in Section 15. If the application did not meet this range, the Study Director must be notified of this deviation before proceeding with this trial.**

The submitted Field Data Book shall contain the original calibration data or a true copy of all calibrations referenced, along with the original data from the rechecks performed for this trial.

15. APPLICATION TREATMENTS AND TIMING:

15.1 These treatments shall be applied in all trials:

Trt#	Treatment	Target Rate of active ingredient	Target Rate of formulated product*	Application Type	Spray Volume Range**
01	Untreated	Not Applicable	Not Applicable	Not Applicable	Not Applicable
02	ETHEPHON	0.75 lb ai/acre (300 ppm)	473 ml/acre (no adjuvant permitted)	Foliar directed	300 GPA (+/-10%)

*The nominal formulation concentration of the test substance will be used in calculating application rates (see Section 13 for the nominal concentration).
**GPA=gallons per acre
If it appears that phytotoxicity has resulted from applications made in this trial, contact the Study Director. If possible, take one or more photographs and send them to the Study Director via email to facilitate the evaluation of crop/ test substance effects.

All trials except Decline Trial 12754.20-FL145: Make one foliar application at 7 (±1) days before harvest.

Decline Trial 12754.20-FL145: Make one foliar application on the first day of multiple sample collections.

16. SUPPLEMENTAL CROP TREATMENTS:

Protect the integrity of the field trial by managing pests that may cause significant damage to the test crop. Only EPA-registered maintenance pesticides should be used; apply according to labeled directions. Make identical applications to the untreated and treated plots. In a field trial with multiple sample collection dates for the treated plot, maintenance applications may be made on that treated plot that are not made on the untreated plot or other plots from which sample collection has been completed.

Consult with Study Director if no registered pesticides are available to control the pests. Document all supplemental crop treatments. DO NOT USE pesticides that are similar to the test substance or other chemicals that might interfere with analysis of the test substance. If unsure, **contact the Study Director**.

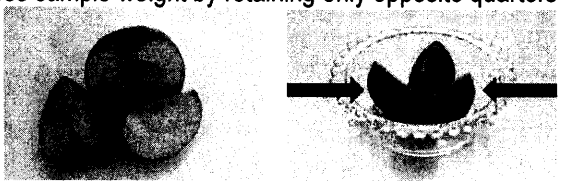
17. RESIDUE SAMPLE COLLECTION:

All trials except decline trial: Collect two samples from each plot. Each sample should be representative of the entire plot (except plot ends). At 7 (±1) days after the last application, starting with the untreated plot, collect a minimum of 24 fruit per sample from at least 4 trees. At least one fruit from each tree should be impartially picked from high, low, sheltered and exposed throughout the treated plot excluding the end trees. Each sample should be collected during a separate run through the entire plot. Avoid sampling from plot ends.

Decline trial 12754.20-FL145 only: (See sample inventory in Protocol Section 18): Follow the sample collection directions noted above except collect only those samples listed in Section 18 at 0, 3 (± 1), 7 (± 1), 14 (± 1) and 21 (± 2) days after the last application. The untreated samples should be collected at the 0 day schedule before collecting the

treated samples. The untreated samples may be collected prior to handling the test substance on the day of the last application. Collect the 0 day treated samples after the spray has dried. Document in the field data book that the spray has dried after the application.

All trials: Each sample should weigh a minimum of 4 lb (but preferably not more than 8 lb). Cut each fruit longitudinally into quarters with a clean knife on an uncontaminated surface. If the sample weighs less than 8 lb, then retain all four quarters. Otherwise, reduce gross sample weight by retaining only opposite quarters from each fruit for the sample.



Longitudinal cuts / Opposite quarters

Process untreated sample first. Record the length of time from completion of the sample reduction to placement in a cooler for each sample in Field Data Book Part 7.A.2.

Follow proper handling practices with clean or gloved hands and clean tools to prevent transfer of pesticide residue from one sample to another. If practical, complete harvest and sample preparation from the untreated plot(s) before proceeding to the treated plot(s).

Place all samples in plastic-lined cloth bags. Bags may be obtained from the Field Research Coordinator (See Section 23). Identify each sample bag** with correct Field ID number, Test Substance (chemical name listed in Section 15), complete sample ID (see Section 18.1) and harvest/sampling dates. After residue sample collection, store samples in a freezer. If the samples cannot be placed into a freezer within one hour, use an appropriate method of cooling and temperature-monitoring samples in order to maintain integrity.

When using **IR-4 plastic lined cloth residue sample bags, complete attached sample tag as follows:
Field ID Number; Crop Fraction; Test Substance (enter the chemical name listed in Section 15); **Sample ID; Trt#; Harvest Date; Sample Date; Field Research Director** (enter name and telephone number).

For Fresh Orange Fruit to be Processed into Dried Pulp, Juice and Oil (Field Trial 12754.20-FL146): Harvest approximately 400- 425 lb of oranges from the Trt 01 plot and the Trt 02 plot at 7 (±1) days after the last test substance application. These samples will be processed into dried pulp, juice, and oil. For processing, oranges should be harvested as per commercial practice. At least one fruit from each tree should be impartially picked from high, low, sheltered and exposed throughout the treated plot excluding the end trees. Avoid sampling from plot ends.

Follow proper handling practices with clean or gloved hands and clean tools to prevent transfer of pesticide residue from one sample to another. If practical, complete harvest and sample preparation from the untreated plot(s) before proceeding to the treated plot(s).

Samples should be collected in a container or containers suitable for shipment to the processing laboratory. Identify each sample with correct Field ID Number, Test Substance (chemical name listed in Section 15) complete sample ID (See Section 18.3), and harvest/sampling dates. See Protocol Section 19.2 for handling and shipping directions. Ship the large samples as "fresh" samples, within 1 day of collection, to the processing facility.

18. FIELD RESIDUESAMPLE INVENTORY:

18.1 All field trials except Decline Trial 12754.20-FL145:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.	MINIMUM SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	24 fruits / 4 lb.	Fruit
B	01	Untreated	NA	24 fruits / 4 lb.	Fruit
C	02	ETHEPHON	7 (±1)	24 fruits / 4 lb.	Fruit
D	02	ETHEPHON	7 (±1)	24 fruits / 4 lb.	Fruit

18.2 Decline Trial 12754.20-FL145:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC	MINIMUM SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	24 fruits / 4 lb.	Fruit
B	01	Untreated	NA	24 fruits / 4 lb.	Fruit
E*	02	ETHEPHON	0	24 fruits / 4 lb.	Fruit
F*	02	ETHEPHON	0	24 fruits / 4 lb.	Fruit
G*	02	ETHEPHON	3 (± 1)	24 fruits / 4 lb.	Fruit
H*	02	ETHEPHON	3 (± 1)	24 fruits / 4 lb.	Fruit
C	02	ETHEPHON	7 (±1)	24 fruits / 4 lb.	Fruit
D	02	ETHEPHON	7 (±1)	24 fruits / 4 lb.	Fruit
I*	02	ETHEPHON	14 (±1)	24 fruits / 4 lb.	Fruit
J*	02	ETHEPHON	14 (±1)	24 fruits / 4 lb.	Fruit
K*	02	ETHEPHON	21 (±2)	24 fruits / 4 lb.	Fruit
L*	02	ETHEPHON	21 (±2)	24 fruits / 4 lb.	Fruit

*Sample IDs are out of sequence in order to maintain consistency among trials for Samples C and D.

18.3 PROCESSING RESIDUE SAMPLE INVENTORY: Trial 12754.20-FL146 only

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.	APPROX. WGT. OF SAMPLE	CROP FRACTION
PA	01	Untreated	NA	440- 425 lb.	Fresh Fruit
PT	03	ETHEPHON	7 (±1)	400- 425 lb.	Fresh Fruit

19. RESIDUE SAMPLE HANDLING AND SHIPMENT:

See below for instructions for handling residue samples (not for processing) that are to be sent directly to an analytical laboratory and instructions for handling processing samples that are to be sent to a processing facility.

19.1 RESIDUE SAMPLE HANDLING AND SHIPMENT: (Samples not for processing)

The methods used in harvest, sample handling, and storage will be outlined generally in SOP's, and described fully in the Field Data Book.

For pre-shipment storage, the samples will be held frozen at temperatures generally less than -20 °C (0 °F), allowing for normal variations of less than 24 hours' duration due to freezer cycling, sample movement, etc. Freezer logs will be used to document all sample additions to and removals from storage. All on-site storage temperatures will be monitored and documented. If the analytical laboratory is close enough to the field site to permit delivery of the samples by field personnel on the day of sampling, then pre-shipment frozen storage is not required.

For express shipments (overnight carriers such as Federal Express or Airborne), contact the designated person (noted below) from the analytical laboratory prior to sample shipment for any specific shipping instructions. **For shipments via freezer truck (ACDS), it is acceptable to contact the laboratory on the day before or the day of shipment, before or after the samples have been loaded on the truck.** Shipment of frozen samples will be by freezer truck or express shipment, unless the samples are brought to the analytical laboratory by field trial personnel. Shipments sent via express shipment (overnight carriers such as Federal Express or Airborne) will require the addition of quantities of dry ice sufficient to maintain sample integrity while in transit to the laboratory (see IR-4 Advisory 2007-01 for more information). If field trial personnel transport the samples to the analytical laboratory directly from the plots and the sampling-to-freezer interval is more than one hour, an appropriate method of cooling and temperature-monitoring shall be used to maintain sample integrity. If the samples are stored frozen at the field trial facility prior to being transferred to the analytical laboratory by field trial personnel, then appropriate methods must be used to keep the samples frozen during transport. These methods should be documented in the FDB.

Document the notification made to the sample destination by use of e-mail, fax, telephone log, Field Data Book communication note, etc.

Insert a true copy of Field Data Book Part 8B and a blank copy of Field Data Book Part 8C (Sample Arrival Check Sheet) into each box or container used to ship sample bags. This documentation is needed even when field personnel transport the samples to the analytical laboratory. Send samples to: Bronson Hung, IR-4 Western Region Laboratory, Center for Health and the Environment (CHE), Univ of California, Davis, Building 3792, 1250 Old Davis Road, Room 129, Davis, CA 95616-8615 (530) 752-4742, FAX# 530-752-5857; e-mail: bkhung@ucdavis.edu

19.2 PROCESSING SAMPLE HANDLING AND SHIPMENT:

Contact the processing lab as soon as you know the date you expect to ship the large, fresh samples for processing, so that the lab will be ready to receive them and begin the processing part of the study as needed. If samples for processing are not shipped to the processing facility on the day of harvest, they should be stored in a refrigerator at approximately 4°C until they are shipped. At the time of shipping large samples, contact the processing lab (document this communication in the field data book). **Insert a true copy of Field Data Book Part 8B and a blank copy of Field Data Book Part 8C (Sample Arrival Check Sheet) into each box or container used to ship samples.** Send samples for processing to: Joshua Bevan, Director, University of Idaho Food Technology Center, 1908 E. Chicago Street, Caldwell, ID 83605; (208) 795-5332; FAX# 208-795-5338; e-mail: jbevan@uidaho.edu

19.3 PROCESSING:

Immediately prior to processing oranges, remove a representative 24-fruit sample (approximately 4-6 lb. for each sample) of oranges from the untreated and treated samples, and immediately place in the freezer.

Using simulated commercial processing (provide detailed description of equipment and procedures), produce dried pulp, juice and oil from the untreated and treated samples. Process untreated fruit first, followed by treated fruit. From the untreated fruit collect one sample each of juice, dried pulp, and oil, using all available fruit after removing the 24-fruit sample. From the treated fruit collect one sample each of juice, dried pulp, and oil, using all available fruit after removing the 24 fruit sample.

Place samples in appropriate containers and label. **Divide each sample of juice and oil into separate containers of 50-150 grams. It is also acceptable to divide dried pulp samples into multiple containers. Each portion of the divided sample should be representative of the whole sample.** Identify each sample with correct Processing ID number, Test Substance (common chemical name), complete sample ID (see table in this section), and processing date(s).

Processed samples should be frozen as soon as possible after processing is completed. For these samples, the processing lab should follow Protocol Section 19 procedures required for freezing/shipping samples to the analytical lab.

If processing cannot take place within 3 days of sample collection, then the samples should be stored in typical orange storage conditions to prevent test substance residue degradation.

Maintain all frozen samples at temperatures generally less than –18 °C (0 °F) until shipped, allowing for normal variations of less than 24 hours' duration due to freezer cycling, sample movement, etc. Freezer logs will be used to document all sample additions to and removals from storage. All storage temperatures are to be monitored and documented.

Contact the designated person (noted below) from the analytical laboratory prior to shipment of samples for any specific shipping instructions. Shipment of frozen samples will be by freezer truck or "express" shipment. Shipments sent via "express shipment" (overnight carriers such as Federal Express or Airborne) will require the addition of quantities of dry ice sufficient to maintain sample integrity while in transit to the laboratory (see IR-4 Advisory 2007-01 for more information). Document the notification made to the sample destination by use of e-mail, fax, telephone log, field data book communication note, etc. For analysis of processed fractions, send samples to: Bronson Hung, IR-4 Western Region Laboratory, Center for Health and the Environment (CHE), Univ of California, Davis, Building 3792, 1250 Old Davis Road, Room 129, Davis, CA 95616-8615 (530) 752-4742, FAX# 530-752-5857; e-mail: bkhung@ucdavis.edu

19.4 PROCESSED SAMPLE INVENTORY:

SAMPLE ID	TRT#	TREATMENT	APPROX. WGT. OR VOL. RANGE OF SAMPLE	CROP FRACTION
GA	01	Untreated	4-6 lb	Whole fruit, just prior to processing
GT	03	ETHEPHON	4-6 lb	Whole fruit, just prior to processing
DPA	01	Untreated	All available	Dried pulp
DPT	03	ETHEPHON	All available	Dried pulp
JA	01	Untreated	All available	Juice
JT	03	ETHEPHON	All available	Juice
OA	01	Untreated	All available	Oil
OT	03	ETHEPHON	All available	Oil

20. FIELD DOCUMENTATION AND RECORD KEEPING:

All operations, data and observations appropriate to this study should be **recorded directly and promptly** into the IR-4 Field Data Book or equivalent raw data notebook.

The content of the Field Data Book should be **sufficiently detailed to completely reconstruct the field trial.** At a minimum, collect and maintain the following raw data:

20.01- Names of all personnel conducting specific research functions

20.02- Amendments and deviations from protocol and standard operating procedures

20.03- Test site information

20.04- Plot maps

20.05 - Test substance receipt, use and container/substance disposition records

20.06- Test substance storage conditions (including temperatures)

20.07- Data regarding calibration and use of application equipment

20.08- Treatment application data

20.09- Crop maintenance pesticides and cultural practices, test plot history, and soil information. (Reporting soil information from typical farm service soil analysis labs, or past history for the farm, or from official documents, such as the SCS Soil Survey for the test plot area is adequate for this study. The nature of this study is such that soil characteristics do not need to be determined under GLP standards.)

20.10- Residue sample identification, collection, storage conditions and handling (Weight measurements are considered estimates for the samples collected from field or processing trials, and the scales/balances used for this purpose do not need to be maintained in strict adherence to GLP.)

20.11- Residue sample shipping information

20.12- Description of crop destruction, or explanation for lack of destruction

20.13- Daily Meteorological/Irrigation records (temperature/humidity records for greenhouse trials)--required from the date of planting or transplanting of annual crops or for a minimum of one month prior to the first application onto perennial crops, until last residue sample collection. These records do not need to be determined under GLP standards. If the protocol requires that transplants are treated with the test substance prior to transplanting, then weather records are required from the date of seeding. If transplants are used for an IR-4 trial but no test substance applications are made prior to the transplanting, then temperature/humidity records are NOT required for the period prior to transplanting.

20.14- Pass times (if applicable) and other data to confirm amount of material applied to plots

20.15- Equipment maintenance records with indication of routine vs. non-routine nature of maintenance

20.16- Other applicable data requested in the IR-4 Field Data Book necessary for confirmation that the study was conducted in accordance with the protocol.

Compliance with GLP's is not required for the collection of data associated with crop phytotoxicity.

20.1 PROCESSING DOCUMENTATION AND RECORD KEEPING:

At a minimum, collect and maintain the following raw data:

20.1.01- Names of all personnel conducting specific research functions

20.1.02- Deviations from protocol and standard operating procedures

20.1.03- Date fresh orange fruit samples received

20.1.04- Storage temperatures until fresh orange fruit samples are processed into dried pulp, juice, and oil

20.1.05- Processing Methodology (SOPs are acceptable)

20.1.06- Data collected and observations made during processing of samples into dried pulp, juice, and oil

20.1.07- Storage temperatures of orange fruit, dried pulp, juice, and oil until shipped

20.1.08- Date orange fruit, dried pulp, juice, and oil are shipped to analytical laboratory

A processing summary report should be prepared and submitted to the sponsor representative. When the processing summary report is completed the report and all original raw data will be sent to IR-4 Headquarters in Princeton, NJ (when an original document cannot be provided a "true copy" will be provided). All original raw data shall be secured in

the archives of IR-4 Headquarters, Princeton, NJ. A "true copy" of the raw data and the final processing report shall be secured in the archives of the Processing Research Director/Testing Facility.

21. PROTOCOL/SOP MODIFICATIONS - FIELD RESEARCH:

Consult with the Study Director and with the Regional/ARS Field Research Coordinator to discuss desired changes in the protocol prior to occurrence. If appropriate, an amendment will be issued.

Any deviations from the protocol will require the Field Research Director to complete a written report outlining the changes. **Provide this report to the Study Director promptly** (e.g. within 14 days of occurrence or recognition) for review and signature.

All deviations from the approved SOP's also require documentation and **approval by the Study Director**.

22. FIELD RESEARCH REPORT/ARCHIVING:

The Field Research Director will forward the completed originals of the IR-4 Field Data Book and other raw data to the Regional/ARS Field Research Coordinator as soon as possible after the shipment of residue samples.

The Field Research Director will maintain a complete certified true copy of these field documents.

The original IR-4 Field Data Book and other raw data will be forwarded to IR-4 Headquarters for reporting and archiving.

23. FIELD PERSONNEL / ID NO. / REGIONAL/ARS FIELD RESEARCH LOCATION

Field trials will be conducted at the appropriate sites to support the establishment/maintenance of a national residue tolerance. If a Field Research Director is assigned more than one trial in this study, refer to Section 11.4 for requirements to differentiate the trials.

Field Research Director	Field ID NO.	RFC	Test Crop
Darrell Thomas, University of Florida, Plant Science Research & Education Unit, 2556 West Hwy 318, Citra FL 32113-2132; Phone: 352-591-2678 x251; fax: 352-591-9860; e-mail: thomasda@ufl.edu	12754.20-FL141	SOR	Orange
Michael Long, University of Florida, Plant Science Research & Education Unit, 2556 West Hwy 318, Citra FL 32113-2132; Phone: 352-591-2678 243; fax: 352-591-9860; e-mail: michaeljlong@ufl.edu	12754.20-FL142	SOR	Orange
Michael Frost, Florida Pesticide Research, Inc., 1810 Deleon St, Oviedo, FL 32765; Ph: 407-365-5360; e-mail: flpesttom@bellsouth.net	12754.20-FL143	SOR	Orange
Michael Frost, Florida Pesticide Research, Inc., 1810 Deleon St, Oviedo, FL 32765; Ph: 407-365-5360; e-mail: flpesttom@bellsouth.net	12754.20-FL144	SOR	Orange
Michael Frost, Florida Pesticide Research, Inc., 1810 Deleon St, Oviedo, FL 32765; Ph: 407-365-5360; e-mail: flpesttom@bellsouth.net	12754.20-FL145 (Decline)	SOR	Orange
Michael Frost, Florida Pesticide Research, Inc., 1810 Deleon St, Oviedo, FL 32765; Ph: 407-365-5360; e-mail: flpesttom@bellsouth.net	12754.20-FL146 (Processing)	SOR	Orange

RFC = Regional/ARS Field Coordinator

Location:

SOR: Dr. Janine Spies, Univ of Florida, 1642 SW 23rd Drive, Bldg 685, PO Box 110720, Gainesville, FL 32611-0720 Tel: (352) 294-3991, FAX# 352-392-1988; e-mail: jrazze@ufl.edu

23.1 PROCESSING PERSONNEL/ID NO.: **PROCESSING ID NO.:** **12754.20-IDP03**

PROCESSING RESEARCH DIRECTOR/PROCESSING FACILITY:

Joshua Bevan, Director, University of Idaho Food Technology Center, 1908 E. Chicago Street, Caldwell, ID 83605; (208) 795-5332; FAX# 208-795-5338; e-mail: jbevan@uidaho.edu

24. LABORATORY PERSONNEL/ID NO.: **LAB ID NO.:** **12754.20-CAR01**

LABORATORY RESEARCH DIRECTOR/TESTING LABORATORY:

Matt Hengel, University of California, Davis, Western Region IR-4 Project, 4218 Meyer Hall, Davis, CA 95616, (530) 752-2402, FAX# 530-754-8556; cell 530-867-2402; e-mail: mjhengel@ucdavis.ed

25. LABORATORY SAMPLE INVENTORY:

Treated and untreated samples of Orange will be received from each of the field and processing sites in Section 23.

Notify appropriate Field Research Director and Regional/ARS Field Research Coordinator of sample receipt.

26. LABORATORY SAMPLE IDENTIFICATION:

Each sample (raw commodity, crop fractions, storage stability, method validation, etc.) is to be assigned a unique laboratory sample number by the laboratory personnel.

A cross-reference must be maintained between the assigned laboratory sample number and the identification utilized in the Residue Sample Shipping and Identification Sheet.

27. LABORATORY SAMPLE STORAGE/PREPARATION:

Store samples in a limited access area at temperatures (generally less than -20°C) that will maintain frozen sample integrity, until extraction.

The samples may be stored whole or ground, depending on the standard procedure of the analytical laboratory. However, if maceration will cause residue deterioration, then samples must be stored whole until analysis.

Do not composite samples.

The entire sample provide from the field must be ground, if sample is too large to be manageable then contact the Study Director for appropriate subsampling to assure the representative nature of the sample obtained in the field is maintained by the laboratory procedure.

Generally, sample extracts should be stored at $\leq$ 4°C for no longer than 14 days before analysis.

Storage stability of extracts must be demonstrated if extracts are not analyzed on the same day as they are obtained.

Concurrent fortifications may be used to show extract storage stability, as long as the extracts from the concurrent fortifications have been stored at least as long as the extracts obtained from the weathered samples.

Contact the Study Director if samples extracts are stored greater than 14 days prior to analysis.

All storage temperatures, conditions and location of sample storage are to be monitored and documented.

28. LABORATORY REFERENCE SUBSTANCE:

Obtain the laboratory reference substance, ethephon, from the Registrant. Contact Sherry Hutcheson, UPL NA Inc., Phone: 229-247-9041, email: sherry.hutcheson@upl-ltd.com to procure the proper material.

Document the date the analytical standards are received, the source, stated purity, storage conditions, and expiration date.

Use only reference standards that have been characterized to meet GLP standards.

Archival and characterization of the reference substance (purity, identity, stability and solubility) is the responsibility of the registrant.

29. ANALYTICAL METHODOLOGY:

REFERENCE METHOD:

"Detailed Method of Analysis for Residues of (2-chloroethyl) Phosphonic Acid (Ethephon) in a variety of Sample Types," Rhone-Poulenc Ag Company SOP – 90070 Issue 1.3, 5/3/1990.

REFERENCE METHOD MODIFICATIONS/METHOD VALIDATION

The above listed Reference Method(s) may be modified if needed for the test matrix.

The Reference Method, along with any modifications must be validated on each crop fraction prior to residue sample analysis of that crop fraction.

To validate the method, fortify some of the control samples in triplicate with ethephon at a minimum of 3 concentration levels, lowest level of method validation (0.02 ppm or lower), 0.2 ppm, and 5 ppm.

A minimum of 6 fortification samples (recovery spikes) at the lowest level of method validation (LLMV) is required for each analyte on each fraction prior to completion of the analytical phase of the study. **The acceptable recovery range is 70-120%.**

Documented approval from the Study Director is needed for recoveries outside of this range.

Document the exact procedures for sample analysis.

This validated step-by-step Working Method should incorporate all changes from the Reference Method.

Provide the Study Director with a copy of this Working Method and results of method validation prior to treated sample analysis.

If the Working Method has been used successfully on the test matrix or a similar matrix, the Study Director may waive the requirement for method validation. **Contact the Study Director for details**.

SAMPLE ANALYSIS:

Samples will be analyzed for the total and/or combined residues of ethephon following the Working Method.

For each field trial associated with this study, analyze at least one untreated and all treated residue samples for each matrix.

Contact the Study Director if residues above the lowest level of method validation for each matrix are detected in the untreated samples.

Any 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 report.

A typical analytical set (or run) should consist of calibration standards, untreated sample(s), concurrent recovery sample(s), and treated sample(s). Each analytical set must begin and end with a calibration standard. Additional calibration standards should be injected with sample analysis to ensure goodness of fit to the standard curve.

Over the course of method validation, residue sample and storage stability (if appropriate) analysis, adequate fortification samples that bracket the actual residues should be analyzed. At least one concurrent fortification sample should be analyzed per analytical set.

The Study Director should be immediately notified 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.

If residues in samples are above the highest Working Method validation concentration, additional recovery samples at levels above actual residues must be run in triplicate (3 uniquely extracted samples) as soon as practical. A minimum of 6 fortification samples (recovery spikes) at the lowest level of method validation (LLMV) is required for each analyte on each fraction prior to completion of the analytical phase of the study.

Treated samples may be analyzed using a screening run prior to analysis of treated samples using the working method, if the procedure is covered in the laboratory SOPs and the working method for the study. The peak areas of the treated samples and highest standard from any screening run will not be quantified or reported. (Any data, such as chromatograms, generated during screening run(s) will be kept.)

STORAGE STABILITY ANALYSIS:

As soon as possible after receipt of samples, a minimum of six subsamples of all available crop fractions of the control shall be fortified ethephon at 0.2 ppm.

Contact the Study Director after the analysis of treated samples has been completed. Only if required by the Study Director at that point, three samples of each analyte and crop fraction will be analyzed after the appropriate storage period. The analysis of storage stability samples may be conducted following a storage period equal to or greater than 90% of the longest storage period of the field –treated samples from collection in the field/processing facility until their analysis. The remaining samples will be

retained for long-term storage. If analysis of treated/control samples is completed within 30 days of harvest, analysis of storage fortification samples may not be required. If appropriate, contact Study Director.

STATISTICAL METHOD(S):

Utilize regression analysis to determine the linearity of the standard curve (r^2) or the goodness of fit if the standard curve is non-linear.

Criteria for acceptance of the standard curve(s) or other statistical methods shall be determined by Laboratory Research Director and documented in the raw data.

30. DISPOSITION OF SAMPLES:

A minimum of 100 g or all (if less than 100 g) of each of the remaining frozen treated and untreated crop samples is to be retained for at least 12 months after submission of the laboratory report.

Long term fortified storage study samples shall be retained for a period of 1 to 5 years, as appropriate, after submission of the final report.

Sample extracts can be disposed of after data analysis.

The Study Director is to be contacted prior to discarding samples.

31. LABORATORY PROTOCOL/SOP MODIFICATIONS - LABORATORY RESEARCH:

Consult with the Study Director regarding desired changes in the protocol prior to occurrence. If appropriate, an amendment will be issued. Any unauthorized changes to the protocol will require the Laboratory Research Director to complete a written report outlining the changes.

This report should be **provided to the Study Director promptly** (e.g. within 14 days of occurrence) for review and signature.

All deviations from the approved SOP's also require documentation and approval by the Study Director.

32. LABORATORY DOCUMENTATION AND RECORD KEEPING:

All operations, data and observations shall be recorded in the analyst's notebook and log books, which must be signed and dated on date of entry.

At a minimum, collect and maintain the following raw data:

32.01 - Analytical standard(s) receipt, use and disposition records

32.02 - Analytical standard(s) storage conditions

32.03 - Analytical standard(s) dilution calculations and preparation records

32.04 - Sample storage conditions and locations

32.05 - Calculation work sheets

32.06 - All chromatograms, including those that are not reported

32.07 - Chain of custody records

32.08 - Deviations from protocol, Working Method and/or standard operating procedures

32.09 - Name of personnel conducting specific research functions

32.10 - Sample analysis worksheets

32.11- Storage stability fortification records

32.12 - Concurrent recovery fortification records

A study file shall be developed and maintained by the Laboratory Research Director in conjunction with the analysis. It will contain a copy of the protocol, all pertinent raw data, documentation, records, correspondence, and the final analytical summary report. In addition, records of equipment maintenance and calibrations will be kept and periodically archived.

33. LABORATORY RESEARCH REPORT:

The analytical summary report sent to IR-4 HQ shall contain, but not be limited to:

33.01 - Applicable method validation data

33.02 - Applicable storage stability data

33.03 - Residue levels for control samples, treated samples, and concurrent fortified recoveries

33.04 - Complete copy of the analytical Working Method

33.05 - Any modifications or deviations from the protocol and/or Working Method

33.06 - A minimum of 10 representative chromatograms of treated samples (if fewer than 10 submit all), a minimum of three chromatograms each of control and fortified control samples, chromatograms (one of each concentration) for at least one set of calibration standards for each compound analyzed, and any chromatograms of samples with unusual or inconsistent results

33.07 - Summary of quantitative data associated with samples and spike recovery samples should be provided (e.g. peak heights, injection volumes, sample sizes, final volumes, etc.)

33.08 - Clearly presented example calculations or statistical evaluations

33.09 - Discussion of results (including purpose of method modifications, sample storage conditions, etc.)

33.10 - Summary data associated with calibration standards (dilution and use records, calibration curves, etc.)

34. LABORATORY ARCHIVES:

For studies assigned to the IR-4 Laboratory at the University of California (CAR), University of Florida (FLR), or Michigan State University (MIR): When the final analytical summary report is completed and sent to the sponsor representative, all original raw data including a "true copy" of the final analytical summary report shall be secured in the archives of the Laboratory Research Director/Testing Facility.

For studies assigned to any other analytical laboratory: When the final analytical summary report is completed the analytical report and all original raw data will be sent to IR-4 Project Headquarters, at a location to be provided via amendment (when an original document cannot be provided a "true copy" will be provided). All original raw data shall be secured in the archives of IR-4 Headquarters. A "true copy" of the raw data and the final analytical report shall be secured in the archives of the Laboratory Research Director/Testing Facility.

IR-4
FIELD DATA BOOK

TITLE: ETHEPHON MAGNITUDE OF THE RESIDUE ON ORANGE

PR# 12754

SPONSOR
IR-4 Project Headquarters
500 College Road East, Suite 201 W
Princeton, NJ 08540
(732) 932-9575, FAX# (609) 514-2612

STUDY DIRECTOR
Raymond Leonard
Phone extension 4624
leonard@njaes.rutgers.edu