1. PROJECT TITLE: FLUOPYRAM: Magnitude of the Residue on PAPAYA

2. JUSTIFICATION AND OBJECTIVES:

The IR4 project and the Global Minor Use Foundation (GMUF) has received a request to support residue data generation to establish a registration for FLUOPYRAM in papaya in Peru for control of black spot (*Asperisporium caricae)*, as part of the IICA tropical fruits residue project and, also, to establish a Codex Maximum Residue Limit (MRL).

To establish a MRL on fluopyram, it is required that the magnitude of the residue in or on the commodity be determined as per OECD Guidelines or 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 to the JMPR and EPA with residue chemistry data to support a pesticide tolerance.

To determine the magnitude of residues of total fluopyram in or on papaya, 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) or under OECD GLPs.

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.

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

Humberto Reyes Cervantes
Director of Agri-Food Safety Division
National Agrarian Health Service of Peru (SENASA)
Av. La Molina No. 1915, La Molina, Lima 12, Peru
Office: (511) 313-3300 ext.1405
Cell: (511) 9439-50979
ereyesc@senasa.gob.pe

4. STUDY DIRECTOR [1]:

Javier Aguilar Zapata
Agri-Food Safety Specialist
National Agrarian Health Service of Peru (SENASA)
Av. La Molina No. 1915, La Molina, Lima 12, Peru
Office: (511) 313-3300 ext. 2163
Cell: (511) 9816-09600
jaguilar@senasa.gob.pe

5. PROPOSED DATES:

Experimental Start :	Dec. 2018
Experimental Termination:	Mar. 2018
Study Completion:	Dec. 2019

6. PROPOSED TEST SITES:

Field sites: Refer to Section 23
Laboratory: Refer to Section 24

7. STUDY AUTHORIZATION:

Humberto Reyes Cervantes (Sponsor Representative) Date

Javier Aguilar Zapata (Study Director) Date

[1]In case the Study Director is not available, contact: Humberto Reyes, office (511) 313-3300 ext.1405, cell: (511) 9439-50979; ereyesc@senasa.gob.pe or Joe DeFrancesco in USA ,cell: 541-829-4950, joedefran@gmail.com

7.1 STUDY DIRECTOR INITIALS: _____

7.2 PROTOCOL QUALITY ASSURANCE/ PROTOCOL REVIEW

This protocol has been reviewed by the quality assurance unit

_____

Quality Control Assurance (QA) for Field Date
Javier Vasquez Castro
La Molina Agricultural University
Department of Entomology
Av. La Molina s/n, Lima 12, Peru
Office: (511) 614-7800 ext.397; Cell: (51) 9775-29301; jaque@lamolina.edu.pe

8. GOOD LABORATORY PRACTICE COMPLIANCE:

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 or Organization for Economic Cooperation and Development (OECD) Series on Principles of Good Laboratory Practice and Compliance Monitoring. 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.

9. QUALITY ASSURANCE:

Quality Assurance duties and responsibilities will be in conformance with 40 CFR 160.35 or as outlined in OECD Series. 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:

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

E3Field trials will be conducted at the appropriate sites to support the establishment/maintenance of a national 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. 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. Each plot shall consist of at least 8 trees, if the test substance is to be applied while moving straight down the row. If each tree is to be treated while encircling the tree during the application, then only 6 trees are necessary in each plot (because there are no “end trees”). See Parts 17 & 18 for requirements for residue sampling.

11.2 Employ adequate buffer zones (minimum 15 meters) between each of the plots to prevent contamination but at least 30 meters is strongly preferred, especially if using a mist blower or airblast sprayer for the application. When plants are used as a buffer between the untreated and treated plots, a shorter 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 (fluopyram) use is not registered on this crop, 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, as indicated by national legislation.

11.4 When a Field Research Director (FRD) has been assigned more than one trial in this study, or when two or more trials are assigned to different FRDs in a study, the field sites must be located 32 km (20 miles) apart from each other AND the first harvest of each trial must be separated by at least 30 days. 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. Choosing sites from different representative regions within the country is preferable. An independently prepared tank-mix must be used in each trial.

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 papaya 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.

Fluopyram (Luna Sensation Formulation) must not have been used in or near the plot areas for a minimum of 12 months, and any prior use of the product must be well documented with application date and the rate used.

13. TEST/CONTROL SUBSTANCE:

Use the Luna Sensation formulation of fluopyram (2.1 lbs fluopyram /gallon; 250 g fluopyram/L) (CAS Number 658066-35-4) that has been characterized to meet GLP standards. SFE 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.

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

The registrant will provide a copy of the Certificate of Analysis to the Field Research Investigator and the Study Director. Store the test substance in a secure, clean, dry area and document storage temperatures. Temperature monitoring should begin within 2 days of receipt of the test substance, regardless of where it is held or stored.

Test substance container(s) must be retained until the final study report is completed and submitted to the regulatory agencies or until the Study Director gives permission.

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 national regulations. See shipping documents for

directions for return of the test substance; if none are given, contact the registrant representative: Jessica Fernandez, Bayer Crop Science LP, phone (919) 549-2631, cell: 919-475-1100; e-mail: jessica.fernandez@bayer.com to procure the proper material.

The registrant will archive a retention sample of the test substance. Control substances are not relevant to this study.

14. TEST SUBSTANCE APPLICATION:

14.1 Simulate commercial application practices by applying the test substance in a manner that represents a representative application technique that is used by area commercial growers, while following the directions 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 6 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 hectare). Direct the entire per-hectare 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.

14.2 Full Calibrations for output and speed must be performed to ensure accurate delivery.
Full calibrations for output and speed (time) must be performed to ensure accurate delivery. A full calibration consists of a minimum of three consecutive, documented checks for sprayer output and speed (equipment or walking).

Sprayer Discharge/Output Calibration:
Prior to the first application of the test substance, a complete 3-run calibration of the sprayer is required. Calibration can be the day before application, although it is preferable to calibrate on application day. A full calibration at the test site and on the day of application is preferred. If a full calibration is made one day prior to the application, then a calibration recheck is necessary on the day of application.

If a boom sprayer with individual nozzles is used, record the discharge of each nozzle from each check. Each nozzle discharge should not vary more than 5% from the average discharge of all the nozzles in each individual check. (If an airblast or turbine sprayer is used, it is not necessary to register discharge of individual nozzles.) The variation of the total output recorded for any one of the three checks in a full calibration must not be greater than 5% from the mean of the full calibration. The average of the three checks is considered the sprayer output or discharge rate.

Rechecking the output is necessary for multiple applications, and allowed, as long as application parameters have not changed. A single output check may be conducted to confirm consistent delivery, and must be within 5% of the last complete 3-run calibration, just prior to subsequent applications.

The equipment must be completely recalibrated (3-run calibration) if:

- A recheck results in an output that differs from the mean of the complete calibration by greater than 5%
- The variation of any nozzle's output from the mean output of all of the nozzles during the same run is greater than 5% (this statement does not apply to airblast or turbine sprayers)

To minimize the occurrence of application rates that fall outside the protocol range, calculations for the amount of test substance to be applied that are based on the discharge rate should be performed using mean sprayer output calculated from the most recent complete calibration data (mean of three output checks), not on single-output recheck results.

Verification of the amount of test substance that has been applied will always be calculated using the most recent complete calibration data.

Speed Calibration:
A speed calibration must be performed prior to the first test substance application. Conduct speed calibration in an area adjacent to the test plot, or on similar terrain. Speed rechecks are required for multiple applications on different days. Speed should be recalibrated if a major equipment change has been made.

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:

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	FLUOPYRAM	140 grams fluopyram/hectare	556 ml/hectare + adjuvant ***	Directed spray to foliage and fruit	200-800 L/ha

* The nominal formulation concentration of the test substance will be used in calculating application rates (see Section 13 for the nominal concentration).

** L/ha= Liters per hectar

*** All applications shall include an adjuvant at a rate recommended by the adjuvant label unless the absence of an adjuvant has been chosen to differentiate two trials conducted by the same Field Research Director (see Part 11.4). DO NOT USE AN ORGANO-SILICONE ADJUVANT. Include a copy of the adjuvant label in the Field Data Book.

**** Note that the treated area for directed applications is calculated as row spacing X number of rows X plot length, unless using individual trees.

Make 4 applications at intervals of 7(±1) days with the last application on the day of harvest (0 day PHI).

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.

16. SUPPLEMENTAL CROP TREATMENTS:

Protect the integrity of the field trial by managing pests that may cause significant damage to the test crop. Only 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:

Follow proper handling practices with clean or gloved hands and clean tools to prevent transfer of pesticide residue from one sample to another. Change or clean equipment and gloves between treated and untreated samples.

If the samples cannot be transported from the field to the laboratory for sample preparation (cutting into sections) within approximately one hour, use an appropriate method of cooling samples in order to maintain integrity. To maintain integrity, place samples in an ice chest (cool box) with frozen gel packs to keep samples cool until fruit can be prepared and placed in a freezer. A temperature-monitoring device should be placed in each cooler. The methods used in harvest, sample handling, and storage will be outlined generally in SOPs, and described fully in the field data notebook. Keep treated and untreated samples in separate coolers during transport from field to lab.

No more than 12 hours must pass between sample harvest in the field and when the samples are frozen.

Collect two samples from each plot. Each sample should be representative of the entire plot (except plot ends). On the day of the last application (0 day PHI) (after the test substance has dried), starting with the untreated plot, collect at least 12 fruits (mature green to color break) per sample from the bottom of the fruit column from a minimum of 6 plants. Each sample should be collected during a separate run through the entire plot. No more than two fruits from each plant should be taken. Avoid taking fruits from end plants. (If each tree was treated individually by an applicator who encircled the tree during the application, then there are no end plants to avoid.) To avoid contamination, collect untreated fruit before collecting treated fruit. The untreated samples may be collected prior to handling the test substance on the day of the last application.

If the samples are to be cut prior to freezing, harvest the whole fruit samples into clean plastic bags or other clean covered containers for transport to the laboratory. Mark each bag/container with the Field ID number and treatment number to insure the chain of custody of samples. DO NOT cut the fruit in the field; see Section 19 for instructions.

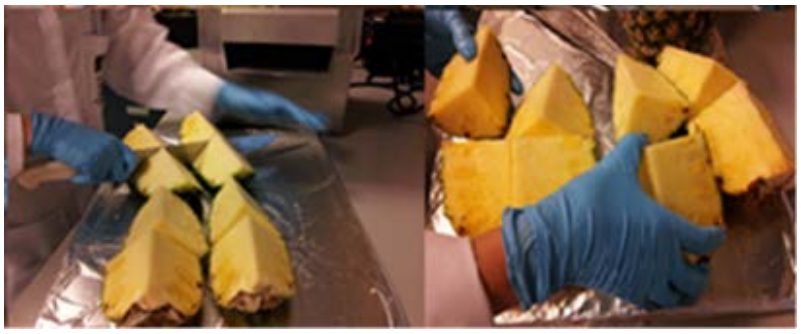
18. FIELD RESIDUE SAMPLE INVENTORY:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.	MINIMUM SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	12 fruits	Non-peeled fruit
B	01	Untreated	NA	12 fruits	Non-peeled fruit
C	02	FLUOPYRAM	0	12 fruits	Non-peeled fruit
D	02	FLUOPYRAM	0	12 fruits	Non-peeled fruit

19. RESIDUE SAMPLE HANDLING AND SHIPMENT TRIAL NUMBER :

If needed to reduce gross weight, cut each fruit into quarters longitudinally and retain opposite quarters for the main sample. If weigh needs to be reduced further, cut each quarter in half and take opposite eighths for the sample. After reducing fruit size, be sure the final weight of each sample is at least 1.8 kg.; try not to exceed 2.7 kg. Do not cut fruit in the field; cut in a clean laboratory space with a clean knife on an uncontaminated surface. Do not peel the fruit. Include a proportional amount of the seeds with each piece of cut fruit.

Longitudinal cuts / Opposite quarters



Or cut in eighths, as above, to further reduce weight of the sample, if necessary

Backup/reserve samples: After taking opposite quarters or eighths for the main sample, keep the remaining quarters or two of the eighths as backup or reserve samples in case the main samples are lost, destroyed or damaged prior to analysis. Label with the sample ID and the word "extra" (e.g. C-extra). If possible, keep the back-up samples in a different freezer from where the main samples are stored. These backup samples do not need be ground, are not to be shipped, and are to be retained in the freezer until the Study Director gives permission to discard them.

Process untreated samples first. Once all fruits from one plot have been prepared, record total weight of the main sample and total weight of the back-up sample in the field data notebook. Also, for each sample, record the length of time from completion of the sample reduction/cutting to placement in the freezer.

Once fruit has been cut and final weight achieved, samples should be placed in a plastic-line cloth bag and frozen immediate. Identify each sample bag with correct Field ID number, Test Substance (common chemical name), complete sample ID code (see Section 18), and harvest date. Back-up/reserve samples may be stored in plastic bags as long as they are properly labeled.

Store samples in a limited access area at temperatures that will maintain frozen sample integrity (generally less than -18ºC), until analyzed or shipped to the analytical laboratory.

Sample handling and storage methods can be outlined generally in SOP's, but describe methods fully in the Field Data Book (FDB).

For pre-shipment and pre-analysis storage, the samples will be held frozen at temperatures generally less than -18°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.

When samples are shipped by air to the analytical laboratory, ensure they maintain their integrity by including dry ice in the shipping containers. If the pineapple was cut into pieces to reduce weight, pack the samples for shipping at a dry ice to sample weight ratio of at least 3:1 (but more ice is better). If samples are ground prior to shipping, even more dry ice is needed to ensure the small (100g) samples do not thaw. Contact the Study Director for instructions prior to shipping ground samples. Place a temperature recording device in each shipping container.

Peru is to ship samples to the analytical laboratory in Costa Rica (see below for shipping address).

To reduce shipping weight, samples from Peru may be ground prior to shipping. Contact the Study Director for approval and guidance on grinding.

Shipment of frozen samples to the analytical laboratory must be coordinated to ensure shipment meets airline standards, dry ice permit regulations, and that someone is waiting for arrival of samples at the airport. Contact the receiving laboratory and document all communication in the field data notebook.

Insert a true copy of Field Data Notebook forms "Residue Sample Chain of Custody" and "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.

For analysis, send samples to: Ms. Karla Arrieta at Servicio Fitosanitario del Estado Headquarter, Laboratorio de Análisis de Residuos de Agroquímicos, 200 m Sur de Canal 7, Sabana Sur, San José, Costa Rica, phone number: (506) 2549- 3424 , cell phone: (506)8846-3853, email: karrieta@sfe.go.cr.

20. FIELD DOCUMENTATION AND RECORD KEEPING:

All operations, data and observations appropriate to this study should be recorded directly and promptly into Field Data Book.

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 (including copies of signed protocol changes received prior to submission of the Field Data Book to the Study Director).
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. 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- Meteorological/Irrigation records--required from planting 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.

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 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.

21. PROTOCOL/SOP MODIFICATIONS - FIELD RESEARCH:

Consult with the Study Director 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 Field Data Book and other raw data to the Study´s Archivist as soon as possible after the shipment of residue samples. Scan or make a copy of the field data book and send it to the study director

The Field Book and all raw data must be reviewed by Quality Assurance before sending to the Study Director.

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

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

23. FIELD PERSONNEL and ID NUMBER:

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.	Test Crop
Luz Huayhua National Agrarian Health Service of Peru (SENASA) Av. La Molina No. 1915, La Molina, Lima 12, Peru Office: (511) 313-3300 ext. 2162 Cell: (511) 9877-69554 Inocuidad02@senasa.gob.pe	12527.18PE-01	Papaya whole fruit

24. LABORATORY PERSONNEL/ID NO.: LAB ID NO.: 12320.18CRLAB-01

LABORATORY RESEARCH DIRECTOR/TESTING LABORATORY:
Ms. Karla Arrieta at Servicio Fitosanitario del Estado Headquarters, Laboratorio de Análisis de Residuos de Agroquímicos, 200 m Sur de Canal 7, Sabana Sur, San José, Costa Rica, phone number: (506) 2549- 3424 , Cell phone: (506)8846-3853, email: karrieta@sfe.go.cr.

25. LABORATORY SAMPLE INVENTORY:
Treated and untreated samples of papaya will be received from each of the field sites in Section 23.
Notify appropriate Field Research Director and Study Director 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 that will maintain frozen sample integrity (generally less than -20 °C), 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 provided 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:

Fluopyram:
Obtain the laboratory reference substance(s) fluopyram (AE C656948) from the Registrant. Contact: Jessica Fernandez, Bayer Crop Science LP, phone (919) 549-2631, cell: 919-475-1100; e-mail: Jessica.fernandez@bayer.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: Fluopyram:

An Analytical Method for the Determination of Residues of AE C656948 in Crop Matrices by Using LC/MS/MS; Bayer Method GM-001-P07-01

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.

Fluopyram:
To validate the method, fortify some of the control samples in triplicate with fluopyram (AE C656948) at a minimum of three concentration levels, lowest level of method validation (0.01 ppm), 0.1 ppm, and 1.0 ppm, in parent equivalents.

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:

Fluopyram:
Samples will be analyzed for the residues of fluopyram (AE C656948) 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:

Fluopyram:
As soon as possible after receipt of samples, a minimum of six subsamples of all available crop fractions of the control shall be fortified with fluopyram (AE C656948) at 0.1 ppm.

Sufficient storage stability data covering the storage intervals of samples for fluopyram (36 months) have been reported by the registrant. Storage stability of fluopyram will be run only if sample storage exceeds the aforementioned storage years reported by the registrant.

Contact the Study Director to determine if storage stability samples need to be analyzed.

Only if directed by the Study Director, 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 Study Director 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 and treated samples with 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 - Completed IR-4 residue data reporting form or appropriate reporting form which includes information listed on the IR-4 generic residue data reporting form
33.07 - 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
33.08 - 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.)
33.09 - Clearly presented example calculations or statistical evaluations
33.10 - Discussion of results (including purpose of method modifications, sample storage conditions, etc.)
33.11 - Summary data associated with calibration standards (dilution and use records, calibration curves, etc.)

34. LABORATORY ARCHIVES:

When the final analytical summary report is completed, all original raw data including a "true copy" of the final analytical summary report shall be secured in the archives of the Laboratory Research Investigator/Testing Facility.