

1. PROJECT TITLE:

OXATHIPIPROLIN: Magnitude of the Residue on CACAO

2. JUSTIFICATION AND OBJECTIVES:

IR4 project and the Global Minor Use Foundation (GMUF) has received a request to support residue data generation to establish minor use a registration of oxathiapiprolin on Cacao for control of Phytophthora spp which cause Black Pod Rot, and, also, to establish a Maximum Residue Limit (MRL).

To establish an MRL, it is necessary to determine the magnitude of residue in and on the commodity be determined according to OECD Guidelines or US-EPA Series 860 Guidelines. The purpose of this study is to collect and analyze treated and untreated samples from appropriate field sites according to the application parameters requested to provide the JMPR and US-EPA with residue chemistry data to support a pesticide tolerance or Codex MRL.

To determine the magnitude of residues of total oxathiapiprolin in or on Cacao, this protocol will be employed using appropriate Standard Operating Procedures (SOP's) and will be conducted under provisions outlined in **IN ACCORDANCE WITH EPA's GOOD LABORATORY PRACTICE STANDARDS** and consistent with the provisions outlined in the Organization for Economic Cooperation and Development (OECD) Series on Principles of Good Laboratory Practice and Compliance Monitoring.

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

Jamie Cardenas Lopez, Assistant Manager of Plant Protection, Colombian Agriculture Institute, National Office Av Calle 26 N° 85B-09 – 7th Floor, Bogota D.C, Colombia. Phone: 57 3323700 ext.1301. E-mail: jamie.cardenas@ica.gov.co

4. STUDY DIRECTOR¹:

Edwin Samir Barbosa-Angel, Colombian Agricultural Research Corporation (Agrosavia), Km 14 Bogotá-Mosquera, Tibaitata Research Center, Phone: 57 311 898 8521, Email: esbarbosa@agrosavia.co

5. PROPOSED DATES:

Experimental Start : September 2019
Experimental Termination: August 2021
Study Completion: September 2022

6. PROPOSED TEST SITES:

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

7. STUDY AUTHORIZATION:

Jamie Cardenas Lopez Sponsor	Date	Edwin Samir Barbosa Ángel Study Director	Date
---------------------------------	------	---	------

7.1 STUDY DIRECTOR INITIALS: _____

7.2 QUALITY CONTROL PROTOCOL/PROTOCOL INSPECTION

This protocol has been reviewed by the lead quality control assurance manager.

Hugo Andres Rodriguez , Quality Control Assurance Colombian Agricultural Institute (ICA) Av. Calle 26 No 42-42 bloque 7 Bogota D.C., Colombia Office: (57 1) 3686827 ext. 5290 Cell: (57) 3115783966 E mail: hugo.rodriguez@ica.gov.co	Date
--	------

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 Good Laboratory Practice (GLP) Standards. A statement of compliance, together with any GLP deviations will be signed and submitted by the appropriate Research Investigators in their report or data package.

9. QUALITY ASSURANCE:

Quality Assurance duties and responsibilities will be in conformance with GLPs. 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:

CACAO - Use a commercial variety when possible. Report: variety, age of plants, etc., if available.

Field trials will be conducted at the appropriate sites to support the establishment/maintenance of a 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 unless otherwise indicated in Section 15. 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".

Field trials #12479.19-CO07 and -CO08 will provide samples for decline trials (multiple sampling dates). The treated plot(s) must be large enough to provide enough samples on each sampling date to meet sample size requirements.

Field trials #12479.19-CO05 and -CO06 will provide extra samples for processing into roasted cocoa beans, cocoa powder, cocoa butter, and chocolate, if needed. The untreated (TRT 01) and treated plot (TRT 03) must be large enough to provide enough samples for processing.

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. 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 The remaining 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 When a Field Research Investigator (FRI) has been assigned more than one trial in this study, or when two or more trials are assigned to different FRIs 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 Investigator 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 Cacao 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:

Use the suspended concentrate formulation of oxathiapiprolin (Syngenta formulation code A21591C) that has been characterized to meet GLP standards. It is known as Orondis Ultra in the USA. This formulation contains 30 g ai/L of oxathiapirprolin and 250 g ai/L of mandipropamid. Syngenta will arrange procurement of the GLP test substance. SYNGENTA will provide a copy of the Certificate of Analysis. Upon receipt, record the lot/batch number, condition, quantity received, and document if it has been GLP characterized. The registrant will archive a retention sample of the test substance. Contact the Study Director if there are any concerns regarding the GLP status, labeled identification, etc., of the test substance.

NOTE: This formulation also contains mandipropamid. Mandipropamid residue data are available in both raw agricultural and processed commodities of cacao at a more critical GAP than will result from use of this formulation according to the use pattern in this study. Therefore, no analyses for mandipropamid will be performed in this study and no further discussion of mandipropamid will be presented in this protocol.

Temperature monitoring should begin within 2 days of receipt of the test substance, regardless of where it is held or stored.

Test substance must be stored in a secure, clean, dry area. Storage temperatures must be documented.

Container(s) with test substance must be stored and preserved until the end of the study, when report has been signed and when the Study Director gives permission.

14. TEST SUBSTANCE APPLICATION:

14.1 Simulate commercial application practices by applying test substance in a way that represents the main application technique used by local commercial producers, while following the directions specified in Section 15.

- Use application equipment that will provide uniform application of the test substance and result in adequate penetration and coverage.
- The test substance, when applied in a mixture with water, must 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.
- Agitate the test substance mixture during the application, if practical, to ensure that it is well mixed.
- For foliar directed applications, do not proportionally reduce the application rate (i.e., the amount of active ingredient applied per hectare). Direct the entire per-hectare rate onto the crop: The treated area for applications is calculated as row spacing X plant spacing X number of trees per plot. If advice is required, contact the Study Director.

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 walking 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 also 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 Field Data Book shall contain the original calibration data or a true copy of all complete 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 Trials 12479.19-CO01, -CO02, -CO03, -CO04, -CO07, and -CO08:

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	OXATHIPIPROLIN (A21591C)	12 grams ai/hectare	400 ml/hectare	Foliar Directed to the trees and fruit.	250-350 L/Ha

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

For trials 12479.19-CO01, -CO02, -CO03, and -CO04: Make 4 applications at an interval of 21(±2) days, with the last application 14(±1) days before harvest. See Sections 17 and 18 for sampling instructions and sample identification.

For DECLINE trials 12479.19-CO07, and -CO08: Make 4 applications at an interval of 21 (±2) days, with the last application 7(±1) days before harvest. See Sections 17 and 18 for sampling instructions and sample identification.

15.2 These treatments shall be applied in Processing Trials 12479.19-CO05 and -CO06:

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	OXATHIPIPROLIN (A21591C)	12 grams ai/hectare	400 ml/hectare	Foliar Directed to the trees and fruit.	250-350 L/Ha
03	OXATHIPIPROLIN (A21591C)	60 grams ai/hectare	2000 ml/hectare	Foliar Directed to the trees and fruit.	250-350 L/Ha

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

For Processing Trials 12479.19-CO05 and -CO06: Make 4 applications at an interval of 21(±2) days, with the last application 14(±1) days before harvest. See Sections 17 and 18 for sampling instructions and sample identification.

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.

Within two days after each application, scan application data from the field notebook, with calculations, and send to Study Director.

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.

Consult with Study Director if no registered pesticides are available to control the pests. Document all supplemental crop treatments. DO NOT USE pesticides containing oxathiapiprolin. If unsure, contact the Study Director.

17. 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. If practical, complete harvest for one plot before proceeding to the next.

Note:

1. For accurate documentation, be aware that "Harvest Date" is the date the cacao fruit was harvested from the plots. "Sample Date" is the date the fermented, dry cacao beans were placed in the sample bags.
2. For planning purposes, it generally takes about 20 cacao pods to produce about 1 kg fermented, dry beans.

For Standard Trials 12479.19-CO01, -CO02, -CO03, and -CO04: At 14(\pm 1) days after the last application, collect two samples from each plot, starting with the untreated plot (TRT 01), then the treated plot (TRT02). Each sample should be collected during a separate run through the entire plot and be representative of the entire plot. Do not harvest fruit from trees at each end of the plot (applicable only if the plots consist of a continuous row of trees).

Place fruits in labeled plastic bags or other suitable containers for transport, keeping each sample separate; make sure each sample is properly labeled with Field ID number, Sample ID number, and date of harvest. Transport samples to a nearby field facility (Agrosavia) where the seeds can be removed from the pods and the fermentation process initiated. Be sure to keep samples separate and labeled during the fermentation process. Fermentation will take place according to standard local procedures (usually about 5-7 days); once fermented, dry the beans as per standard local procedures (about 5-7 days), again, ensuring that samples remain separate and are clearly labeled. Document harvest time and procedures, fermentation, and drying procedures in the Field Data Notebook.

Once cacao beans are fermented and dried, place samples in plastic-lined cloth bags. Identify each sample bag with correct Field ID number, Test Substance (oxathiapiprolin), complete sample ID number (see Section 18) and sampling date (date when fully dried and placed in plastic-lined cloth sample bags).

For Decline Trials 12479.19-CO07 and -CO08 only: Follow the general sample collection procedures as described above. Collect two samples from the treated plot TRT 02 at approximately 3 to 4 day intervals starting at 7(\pm 1) days after the last application, then at 10(\pm 1), 14(\pm 1), 17(\pm 1), and 21(\pm 2) days after the last application. This will result in 10 samples being collected from treated plot TRT 02. **Collect two samples from the untreated plot TRT 01 only at the 14(\pm 1) day sampling event.** Each sample should be collected during a separate run through the entire plot and be representative of the entire plot. Do not harvest fruit from trees at each end of the plot. Follow fermentation and drying procedures as mentioned above.

For Samples for Processing Trials 12479.18-CO05 and -CO06 only: Follow the sample collection procedures as described above. Collect two samples from the control plot TRT 01 and two samples from both treated plots (TRT 02 and TRT 03) at 14(\pm 1) days after the last application. Collect samples starting with the untreated control, then treated plot TRT 02, and lastly treated plot TRT 03. Each sample should be collected during a separate run through the entire plot and be representative of the entire plot. Do not harvest fruit from trees at each end of the plot. Follow fermentation and drying procedures as mentioned above.

It generally takes about 20 cacao pods to produce about 1 kg fermented, dry beans; therefore, to produce the larger samples for processing, at least 100 cacao fruits should be collected.

See Section 19 for residue sample handling directions.

18. FIELD RESIDUE SAMPLE INVENTORY:

Note: It generally takes about 20 cacao fruits to produce about 1 kg of fermented dry beans. The goal is to eventually have 1-2 kg fermented, dry beans for laboratory analysis (samples from the processing trials should yield about 5 kg fermented, dry beans). Based on the cacao variety used in each field trial, plan accordingly and harvest enough fruit pods to yield the required amount of fermented, dry beans per sample.

18.1 For Standard Trials 12479.19-CO01, -CO02, -CO03, and -CO04:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.	MINIMUM FIELD SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	30 Fruits	Whole Fruit
B	01	Untreated	NA	30 Fruits	Whole Fruit
C	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
D	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit

18.2 For Decline Trials 12479.19-CO07 and -CO08 only:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.*	MINIMUM FIELD SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	30 Fruits	Whole Fruit
B	01	Untreated	NA	30 Fruits	Whole Fruit
E	02	OXATHIPIPROLIN	7(±1)	30 Fruits	Whole Fruit
F	02	OXATHIPIPROLIN	7(±1)	30 Fruits	Whole Fruit
G	02	OXATHIPIPROLIN	10(±1)	30 Fruits	Whole Fruit
H	02	OXATHIPIPROLIN	10(±1)	30 Fruits	Whole Fruit
C**	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
D**	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
I	02	OXATHIPIPROLIN	17(±1)	30 Fruits	Whole Fruit
J	02	OXATHIPIPROLIN	17(±1)	30 Fruits	Whole Fruit
K	02	OXATHIPIPROLIN	21(±2)	30 Fruits	Whole Fruit
L	02	OXATHIPIPROLIN	21(±2)	30 Fruits	Whole Fruit

* The intent is to collect these samples at 3 to 4 day intervals.

** Sample IDs are not in alphabetical order so that samples C and D will have the same PHI in all trials.

18.3 For PROCESSING Trials 12479.19-CO05 and –CO06 only:

SAMPLE ID	TRT#	TREATMENT	DAYS AFTER LAST APPLIC.	MINIMUM SAMPLE SIZE	CROP FRACTION
A	01	Untreated	NA	30 Fruits	Whole Fruit
B	01	Untreated	NA	30 Fruits	Whole Fruit
C	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
D	02	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
E	03	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
F	03	OXATHIPIPROLIN	14(±1)	30 Fruits	Whole Fruit
PA	01	Untreated	NA	100 Fruits	Whole Fruit
PB	03	OXATHIPIPROLIN	14(±1)	100 Fruits	Whole Fruit

19. RESIDUE SAMPLES HANDLING AND SHIPPING:

Once samples are fermented and dried, and placed in plastic-lined cloth bags that have been properly labeled with sample ID number, sample date, and other information (see Sections 17 and 18), transport samples to the analytical laboratory, by land or air, keeping samples cool and dry during transport (include frozen gel packs, and a temperature recording device, in the containers). Freeze samples upon arrival in the analytical laboratory.

Samples need to reach the analytical laboratory within 12 hours after the fermentation and drying process is completed.

Sample handling and shipping methods can be outlined generally in SOP's, but methods should be described fully in the Field Data Book.

Contact the designated person (noted below) from the analytical laboratory prior to sample shipment or delivery for any specific instructions. Document the notification made to the sample destination by use of e-mail, fax, telephone log, Field Data Book communication note, etc.

For analysis, send samples to: David Esquivel, Colombian Agriculture Institute-ICA, Km 14 Bogotá-Mosquera. Tibaitatá research center, National agricultural supplies Laboratory-LANIA, Phone: 57+1+ 4227363; E-mail: david.esquivel@ica.gov.co, or to lania@ica.gov.co

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 transports the samples to the analytical laboratory.

19.1 SAMPLE STORAGE at ANALYTICAL LABORATORY:

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. Samples will be stored 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.

19.2 SAMPLES FOR PROCESSING:

Keep the fermented, dry beans samples (PA and PB) from the treated and untreated plots from the processing trials in the freezer. They may not need to be analyzed (results of the residue analysis from the foliar applications

will determine if processing samples need to be analyzed). Do not process the samples until the Study Director gives permission. An amendment will be prepared by the Study Director documenting the instructions for these samples if processing is required.

20. FIELD DOCUMENTATION AND RECORD KEEPING:

All activities, data and observations appropriate to this study should be recorded directly and promptly into the 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 Regional Field Coordinator).
- 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- Daily 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 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.

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 Investigator 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 Investigator will keep the completed originals of the Field Data Notebook and other raw data, and send a scanned copy to the Study Director as soon as possible after the shipment of residue samples.

The Field Data Notebook and all raw data must be examined by Quality Assurance unit (QA) before scanning and sending to the Study Director.

23. FIELD PERSONNEL and ID NUMBER.:

If a Field Research Investigator is assigned to more than one trial in this study, requirements must be taken to differentiate one test from the other (see section 11.4)

Field Research Investigator	Field Identification No.	Test Crop Fraction
Camilo Martinez Agrosavia C.I. Tibaitatá · Mosquera, Cundinamarca Office phone: 4227300 ext 1330 Cell phone: 3118988521 Email: cmartinezr@agrosavia.co	12479.19-CO01	Fermented Dry Bean
	12479.19-CO02	Fermented Dry Bean
	12479.19-CO03	Fermented Dry Bean
	12479.19-CO04	Fermented Dry Bean
	12479.19-CO05	Fermented Dry Bean (PROCESSED)
	12479.19-CO06	Fermented Dry Bean (PROCESSED)
	12479.19-CO07	Fermented Dry Bean (DECLINE)
	12479.19-CO08	Fermented Dry Bean (DECLINE)

Lab and Field Coordinator:

Adriana Castaneda
Phone: +57 313 286 5873
Email: 2018adrianacolombia@gmail.com

23.1 PROCESSING PERSONNEL/ID NO.:

To be determined.....

PROCESSING LAB ID NO.: 12479.19-XXXX

24. LABORATORY PERSONNEL/ID NO.:

David Esquivel
Colombian Agriculture Institute-ICA
Km 14 Bogotá-Mosquera, Tibaitatá research center
National Laboratory for Agricultural Supplies-LANIA,
Phone: 57+1+ 422 73 63
E-mail: david.esquivel@ica.gov.co, lania@ica.gov.co

LAB ID NO.: 12479.19-ICA01

25. LABORATORY SAMPLE INVENTORY:

Treated and untreated samples of FERMENTED, DRY CACAO BEANS will be received from each of the field sites in Section 23. Field Trials 12479.19-CO05 and –CO6 will provide laboratory with enough fermented, dry beans for processing into roasted beans, cocoa powder, cocoa butter, and chocolate (if needed).

Notify appropriate Field Research Investigator 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 -18°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 $\leq 4^{\circ}\text{C}$ 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(s), Oxathiapiprolin, IN-SXS67, and IN-E8S72 from the Registrant. Contact Janet Ruhl, 001-(302) 485-3699, e-mail: janet.c.ruhl@corteva.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: Oxathiapiprolin

The method contained in DuPont Study titled "Analytical Method for the Determination of DPX-QGU42 and Metabolites in Crops Using LC/MS/MS"; Authors: Robert M. Henze and James J. Stry; DuPont Project Identification: DuPont-30422 Supplement No 1.

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 oxathiapiprolin, IN-SXS67, and IN-E8S72 at a minimum of three concentration levels each, lowest level of method validation (0.01 ppm or lower), 0.1 ppm and 1 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%**, but mean recoveries at each level should be 70-110%, with RSD of less than 20%.

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 oxathiapiprolin, IN-SXS67, and IN-E8S72 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.)

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 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 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 Investigator/Testing Facility.