STUDY PLAN AMENDMENT

Study Number: AAFC19-0XXR *Active ingredient* on *Crop*

Study Plan Section(s): 24 to 41

Description of Changes:

Change: Addition of sections 24 through 41 to the Study Plan.

24. LABORATORY PERSONNEL/TRIAL ID NO.:
(Responsible for Sections 25-41)
The Principal Investigator and test site management must sign the GLP Acceptance form (Appendix A) and return as directed.

PRINCIPAL INVESTIGATOR:
The PI will be indicated at a later date and added via an amendment.
The PI must be identified before sample shipment.

TRIAL ID No.
AAFC19-XXXR-XXX

TEST SITE MANAGEMENT:
The Test Site Management will be indicated at a later date and added via an amendment.

The laboratory will be identified at a later date, at which time the appropriate information will be added by amendment.

25. LABORATORY SAMPLE INVENTORY:
Treated and untreated crop samples will be received from the field sites outlined in Section 20 (for responsible persons see Section 10). Notify the appropriate Principal Investigator and Study Director of sample receipt by returning (by fax, email, or mail) a copy of the completed Chain of Custody form or a similar laboratory form used for sample arrival confirmation.

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 (Note, use of the field sample identification number is acceptable). A cross-reference must be maintained between the assigned laboratory sample number and the identification utilized in the Sample Chain of Custody Form received from the field sites. Both identification numbers must be reported in the analytical report.

27. LABORATORY SAMPLE STORAGE/PREPARATION:
Store samples in a limited access area at temperatures that will maintain frozen sample integrity (generally less than –18°C (0°F)), allowing for normal temperature variations [due to freezer cycling, sample movement, etc.] until extraction. The samples may be stored whole or macerated, depending on the standard procedure of the analytical laboratory. However, if maceration will cause residue deterioration, then samples must be stored whole until extraction. Note: The entire sample is to be macerated prior to taking a sample for analysis **and samples are not to be composite.** Contact Study Director if guidance is needed. All storage temperatures, conditions, and location of sample storage must be monitored and documented.

Upon receipt of the samples and reference item(s), using a macerated control sample, prepare and freeze four provisional sets of samples (three fortified samples plus four unfortified samples per set) fortified at the targeted highest concentration of method validation, *(ex.10 ppm)*. These provisional sample sets will be analysed **only** if there is a delay for frozen storage stability analysis as outlined in Section 34. Should analysis of the provisional sample sets be necessary, the samples will be analysed at two time points: 1) as soon as possible after method validation is completed and approved by Study Director, and 2) at a time to cover the period from the first harvest date of the study to the last date of sample analysis.

28. ANALYTICAL SPECIFICATIONS:

- **Residue Definition** – active ingredient and metabolites (e.g. Clethodim, 5-OH-Clethodim Sulfone)
- **Analyte Definition** – active ingredient/ metabolites or conversion products (e.g. DME Sulfone, DME-OH Sulfone)
- **Internal Standards** – (e.g. Glufosinate Hydrochloride-methyl-d_3, Propanoic Acid-methyl-d_3, and N-Acetyl Glufosinate-methyl-d_3)
- **Crop fractions/Matrices** – (e.g. hemp seed, hemp oil and hemp flour)
- **Lowest Level of Method Validation (LLMV)** = (e.g. 0.01 ppm)

29. LABORATORY REFERENCE ITEM(S):

Laboratory reference item(s), *active ingredient(s)* (and any required *metabolites* and/or *internal standards*) obtained from the Registrant are to be used, unless otherwise approved by the Study Director. If required, to procure the reference item(s), contact: *name, company* at phone: *(XXX) XXX-XXXX*, email: *name@company,* and document the request in the raw data. Document the date the reference item(s) is received, the source, lot number, stated purity, storage conditions, and expiration date. Use only reference item(s) that have been characterized to meet GLP standards. Contact the Study Director if there are any concerns regarding the GLP characterization, label identification of the reference item (e.g., the name on the bottle or certificate of analysis (CoA) is different from the Study Plan), etc. and if the reference item does not come with the CoA. Characterization of the reference item(s) (purity, identity, stability, and solubility) and maintenance of an archival sample is the responsibility of the registrant unless otherwise specified by the Study Director.

30. REFERENCE METHOD:

Reference method to be indicated.

If modifications to the reference method are necessary to analyze the specific crop fraction(s), a working method is to be prepared. The working method is to provide all necessary steps in the analysis, including instrument conditions, and is to have a section outlining the need for major modifications from the reference method.

Note: any MS method must have a minimum of two MRM transitions for each analyte, one for quantification and one for confirmation.

Provide the Study Director with the information specified in section 39, prior to method validation.

31. CALIBRATION STANDARDS PREPARATION:

Unless approved by the Study Director, calibration curves are to be comprised of a minimum of five calibration standards prepared from a) at least two different stock solutions (i.e., individually prepared from different weighing of the reference item) and injected in alternation[1] or b) a single stock solution that has been verified against the concentration of a second stock solution. Calibration standards response **must bracket**: the lowest and highest levels of method validation samples; the analyte response of the fortification samples; treated samples with residues above the LLMV; and (if applicable) method validation extension samples. The use of a zero standard[2] or blank as part of the calibration series is not acceptable. A calibration curve is to be generated for each analyte listed in the analyte definition using solvent based calibration standards. Use of matrix matched calibration standards instead of solvent based calibration standards must be experimentally justified during method validation and approved by Study Director for further use.

32. ANALYTICAL SETS:

Analytical system must be equilibrated/conditioned before the start of an analytical run to ensure that the entire system is suitable for analysis. If conditioning injections are included as part of a sequence, they must be clearly designated as conditioning runs. In each analytical run, the solvent blank(s), reagent blank(s) and matrix blank (if matrix matched standards are used) must be run before the first set of calibration standards. In addition, a solvent blank must be run immediately after the highest calibration standard or the highest level fortification sample, to ensure the analysis is free of carryover and/or interferences. The complete calibration set (all calibration standards used to prepare the curve) must be injected before the first and after the last sample. Additionally, calibration standards must be interspersed during the analytical run to ensure goodness of fit to the calibration curve. The acceptable backcalculated[3] concentrations for injected calibration standards are to be within ±20% of the respective theoretical concentrations. Values outside of this range must be justified and sent to the Study Director.

Each injection set (including those with re-injected or diluted sample extracts) should include calibration standards, control (untreated) sample(s), fortified sample(s), a reagent and solvent blank, and if applicable, treated samples.

All field and fortified sample injections must be made in duplicate. The difference in response between duplicate injections is to be ≤10%, otherwise the sample is to be re-injected in duplicate. The mean residue value from the two acceptable injections is to be reported and used in all subsequent calculations. If the re-injection fails, the issue must be investigated. If responses of the duplicate injection are both below the lowest calibration standard, re-injection is not required.

33. METHOD VALIDATION:

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[1] For example, (X, O, X, O, X) but not (X, X, X, O, O), where X is the calibration standard prepared from one stock solution and O is the calibration standard prepared from a second stock solution.

[2] A zero standard is a calibration solution containing no analyte of interest (may contain the internal standard).

[3] Backcalculated means determining the concentrations of the calibration standards using the regression equation. The calculated concentration of each calibration standard is then compared to the actual concentration using the formula: *observed concentration/theoretical concentration x 100*

The method must be validated for each compound in the Residue Definition, for each crop fraction, by using either store purchased (preferably organic) crop fraction or using one of the untreated field samples. To validate the method(s) analyze a minimum of one control (untreated) sample and three replicate fortifications, at each of the following levels: **LLMV, 2X LLMV and 10X LLMV**. The minimum number of validation samples will be 10. The acceptable recovery range is 70-120% and %RSD ≤20%. Documented approval from the Study Director is needed for all recoveries outside of this range.

Document the working method with "stopping points" that will be used for sample analysis. This validated step-by-step working method must outline all changes from the reference method.

Method LOD and LOQ:
As part of the method validation, it must be possible to calculate the method LOD and LOQ for each analyte in each matrix. Either the LOD and LOQ needs to be determined using a method approved by the SD or a minimum of six fortified control samples at LLMV need to be analyzed and used to calculate LOD and LOQ.

Method Validation Extension:
During sample analysis, if residue levels are greater than the highest concentration validated, the method validation needs to be extended. As soon as practical, analyze three replicates of a control (untreated) sample fortified at a concentration above the highest level of residues found in the treated samples (for further guidance see section 35). A solvent blank is to be analyzed after the highest fortified sample. The acceptable recovery range is 70-120% and %RSD ≤20%. Documented approval from the Study Director is needed for all recoveries outside of this range.

Statistical Method(s):
Unweighted linear regression (y=mx +b), which is not forced through the origin, is to be used to generate calibration curves, unless otherwise approved by the Study Director. Calibration curves will have the coefficient of determination (r^2) ≥ 0.99 and backcalculated calibration standards concentrations are to be within ±20% of the theoretical concentration. In general, data points for calibration curves will not be dropped, unless justification is sent to and approved by the Study Director.

Provide the Study Director with the information specified in section 39, for Study Director's approval, prior to sample analysis.

34. STABILITY ANALYSIS:

Stability of Standard Solutions (stock, intermediate/working, and calibration solutions):
Standard solution stability is the stability of a compound, in a unique solvent and storage condition combination, for a defined period of time. *Sentence about known stability in solvent and storage conditions either from the reference method or another study/registrant (ex. Clethodim in acetonitrile at refrigerator temperatures*[4] *is stable for 21 days).* If standard solutions for compounds, identified in the residue and analyte definitions, are not prepared and stored as stated above or are not prepared fresh daily[5] and unless documentation of standard stability is provided and approved by the Study Director, standard stability needs to be conducted.

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[4] Refrigerator refers to storage temperatures of generally 2 to 9°C, with normal variations due to door opening, etc.

[5] Freshly prepared standard – standard prepared from a new weighing of RI on the DATE of comparison

This is to be done by analyzing a solvent/reagent blank, to ensure there is no interference, and then comparing the average response factor (a minimum of five replicate injections) of the aged standard solution (aged for the longest time period the standard was used in the study) to the average response factor (a minimum of five replicate injections) of a freshly prepared[5] standard solution. The compound will be considered stable in solution if the response factor of the aged standard is within ±10% of the freshly prepared standards. Values outside of this range may require re-analysis as determined by the Study Director or Principal Investigator.

Stability of Analytes in Stored Extracts:
All extracts should be stored in a refrigerator[6]. Stability of analytes in stored extracts must be demonstrated if extracts are not analyzed within 24 hours of preparation, unless previously determined for longer time frame. Stability is to be conducted in the following way:

- Analyze a set of samples and then age the samples. After a specific period of time re-analyze the sample set. If average results in the first analysis (original set) are within ±10% of those in the second analysis (aged set), the extracts will be considered stable.

Other stability testing methods must be approved by the Study Director.

Frozen Storage Stability Analysis:
If extraction of treated and control (untreated) samples is completed within **30 days** of harvest (*enter the time period for which you have frozen storage stability data*), analysis of frozen storage stability samples will not be required.

If frozen[7] storage stability analysis is required it should be set up as soon as possible after method validation. Utilizing a control sample from each crop fraction, samples are to be prepared by fortifying them with each compound in the residue definition at the highest level of method validation. For day 0 (the same day the frozen storage samples are prepared), three freshly fortified samples and one control sample are to be analyzed. For all other timeframes (for each timeframe required plus two contingency sets), place a minimum of three fortification samples and four unfortified control samples in frozen storage. After the appropriate storage period, beginning at 30 ± 5 days and then every 90 ± 10 days thereafter, for each compound per crop fraction, three freshly fortified frozen control samples are to be prepared and analyzed along with an unfortified control sample and three aged fortified samples. The last storage sample is to be analyzed at a time period greater than the longest interval between harvest and extraction [8]. Recoveries of the aged fortified samples are to be compared to the recoveries of the freshly fortified samples. If at any time point the recoveries differ by 20% or more, the results will be immediately reported to the Study Director and another sample set will be analyzed as soon as possible (usually within a week of occurrence). Study Director must also be notified immediately if freshly fortified recoveries deviate from the acceptable recovery range of 70-120% and %RSD of ≤20%.

35. SAMPLE ANALYSIS:
Samples are to be analyzed and reported for all compounds in the Residue Definition, following

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[6] Refrigerator refers to storage temperatures of generally 2 to 9°C, with normal variations due to door opening, etc.

[7] Frozen storage refers to storage of samples at temperatures generally less than -18°C (0°F), ensuring samples remain frozen at all times despite short periods of temperature spikes due to freezer door opening, defrosting cycles, etc.

[8] The final time period may be longer or shorter than the scheduled 90-day interval, as approved by the Study Director.

the successful validation of the working method. The analysis is to be conducted in the same manner as that used for the method validation. **Any modifications to the working method may require method revalidation and must be approved by the Study Director**. Whenever possible, notify the Study Director prior to occurrence. Any modification to the working method must be documented in the raw data and the final analytical report.

For each field trial associated with this study, analyze at least one control (untreated) and all treated residue samples for each crop fraction. Contact the Study Director immediately if residues above 20% of the LLMV are detected in the control samples, for any crop fraction. The Study Director must also be notified immediately if residues in any of the treated samples are higher than the highest level of method validation or if they fall outside of the calibration range.

In addition to the treated samples, at least one control (untreated) sample and a minimum of two concurrent fortification samples, each one at a different level (that bracket the expected residue levels), for each compound in the residue definition for each crop fraction are to be analyzed per analytical set. The Study Director must be notified immediately if concurrent recoveries deviate from the acceptable recovery range of 70-120% and %RSD ≤20%.

Sample extracts with analyte response that exceeds the calibration curve range, will be diluted accordingly, and re-injected in a timely manner. The method validation may also need to be extended (see section 33). Any treated samples with residue level higher than the validated level during the original method validation must be re-extracted and reanalyzed with a fortification above the expected residue level (either during the method validation extension or with a new concurrent fortification).

Provide the Study Director with the information specified in section 39, for Study Director's review and approval.

36. DISPOSITION OF SAMPLES:

A minimum of 100 g, of the remaining frozen treated and control crop samples is to be retained for at least 12 months after the Final Analytical Report is completed. Study Director's approval is required prior to discarding remaining samples from the field or frozen storage stability study. Sample extracts can be disposed of after data analysis.

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

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

38. LABORATORY DOCUMENTATION AND RECORD KEEPING:

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

- Names of personnel conducting specific laboratory functions
- Chain of custody records

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

39. LABORATORY REPORTING TO THE STUDY DIRECTOR

At each reporting phase, at a minimum, a copy of the following documents is to be sent to the Study Director:

Method Validation Preparation:

- Certificate(s) of Analysis for all laboratory reference item(s)
- Explanation of key modifications to the Reference Method
- The proposed working method

Method Validation:

- Working method (including stopping points and any changes if different from method validation preparation)
- Results, including:
    - summary of data;
    - acquisition information;
    - calibration curves with equation for the applied regression and coefficient of determination (r^2);
    - chromatograms of the solvent, reagent blank, standards, fortified samples and control (untreated) sample;
    - calculation worksheets (formula and calculations) including:
        - details of dilution of extracts;
        - backcalculation for calibration standard concentrations;
        - %difference in duplicate injections, mean and standard deviation, %recovery, and %RSD
- Standard solution(s): Worksheets for preparation, storage conditions, dilution calculations and preparation records
- Worksheets for preparation of frozen storage stability samples (if applicable)

Sample Analysis:

- Results, including:
    - summary of data;
    - acquisition information;
    - calibration curves with equation for the applied regression and coefficient of determination (r^2);
    - chromatograms of the solvent, reagent blank, standards, fortified samples, treated and untreated sample;
    - calculation worksheets (formula and calculations) including:
        - residue analysis;
        - details of dilution of extracts;
        - backcalculation for calibration standard concentrations;
        - %difference in duplicate injections, mean and standard deviation,

%recovery, and %RSD

40. FINAL ANALYTICAL REPORT:

The Final Analytical Report sent to the Study Director shall contain, but not be limited to:

- Reference item(s) COA(s) and identity including name, structure, purity, lot number, expiration date, source (manufacturer) and storage
- Cross-reference of sample identification numbers
- Detailed description of sub-sampling, maceration procedures and sample storage
- Modifications to the Reference Method(s) and purpose/justifications of those modifications
- Calibration standards weights and preparation procedures
- Complete copy of the step by step analytical working method
- Detailed description of stock solutions and standard solutions storage (container type, storage description including place and number, temperature range, and any temperature fluctuations)
- Clearly presented example calculations and statistical evaluations
- Discussion of results (method validation, concurrent fortification results, field sample results, stability analysis results) including how study plan requirements were met, and any modifications or deviations from the study plan and/or working method

- Method validation data
- Summary data associated with calibration standards and calibration curves (concentration range, regression type, correlation of x and y)
- Summary of quantitative data associated with fortified samples should be provided (e.g., sample weights, final volumes, injection volumes, peak areas/heights, recoveries, %RSDs.)
- Summary of important experimental dates (harvest dates, sample receipt dates, maceration dates, extraction dates, analysis dates, and number of days between harvest date and extraction date)
- Residue levels for untreated and treated samples
- Stability data of standard solutions and analytes in extracts (if required as per Section 34)
- Frozen sample storage stability data (if required in Section 34)
- Representative chromatograms including the following (Note: a "set" represents an analytical injection run done on a particular day):
    - Calibration standards (for each analyte), include one chromatogram for each concentration level and the corresponding calibration curve for one set. In addition, include one chromatogram from each set at the LLMV.
    - If matrix-matched standards are used, a solvent-based calibration curve as well as one chromatogram at the LLMV must be included for comparison.
    - Method validation (for each compound and crop fraction): one chromatogram for each fortification level used (including method validation extensions).
    - Concurrent recoveries (for each compound and crop fraction): chromatograms showing recoveries at the LLMV, as well as the high level fortification.
    - Controls (for each compound and crop fraction): At least one untreated control (UTC) chromatogram per trial, ensuring these include one UTC chromatogram per set.
    - Treated samples (for each compound and crop fraction): minimum of ten chromatograms (all if less than 10 in the study), depicting representative samples per Trial ID covering each day of harvest and covering each day of analysis (ensuring a mixture of C or D samples, a mixture of E or F, etc.).
    - Blanks: one solvent, one reagent and one matrix blank chromatogram. Additionally, include solvent blank chromatogram that was run after a highest level analytical standard or the highest level fortification sample.
    - Any chromatograms with unusual or inconsistent results.
- Supporting information (acquisition method and run sequences)

41. LABORATORY ARCHIVES:

When the Final Analytical Report is completed, the report and all original raw data (hard copy and a scanned [electronic] copy) will be sent to the Study Director, unless another location is designated by the Study Director. The Principal Investigator/Testing Laboratory will maintain a scan or printed copy of these documents. The original raw data will be secured in the archives of the Sponsor once the study is completed.

Reason for Change: Sections 24 through 41 include information related to the laboratory and analytical method to be used for this study. This information was not available for inclusion in the study plan when it was first issued.

Signatures:

Study Director:	_____ *Study Director*	_____ Date
Test Facility Management/: Sponsor Representative	_____ *Submissions Manager*	_____ Date
Reviewed by: Quality Assurance	_____ *QA*	_____ Date