1. PROJECT TITLE: Norflurazon: Magnitude of the Residue on Clover (Seed Crop)

2. JUSTIFICATION AND OBJECTIVES:

IR-4 has received a request for the minor use of norflurazon on clover (seed crop) for control of buckhorn plantain.

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

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

IR-4 Project Headquarters, North Carolina State University, 1730 Varsity Drive, Venture IV, Suite 210, Raleigh, NC 27606, Telephone #: (919) 515-1552

4. STUDY DIRECTOR[1]:

Christina Dineen, IR-4 Headquarters, North Carolina State University, 1730 Varsity Dr, Venture IV, Suite 210, Raleigh, NC 27606, (919) 515-6596, Email: cdineen2@ncsu.edu

5. PROPOSED DATES:

Experimental Start :	02/24
Experimental Termination:	11/26
Study Completion:	10/27

6. STUDY DIRECTOR INITIALS: CDD

7. STUDY AUTHORIZATION:

Jerry Baron 2/1/24	Christina Dineen 02/01/24
Sponsor Representative / Date	Christina Dineen / Study Director / Date

8. GOOD LABORATORY PRACTICE COMPLIANCE:

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

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

[1]In case the Study Director is not available, contact Dr. Jerry Baron at (908) 627-4213 or Dr. Deborah Carpenter at (215) 913-5931 for guidance.

9. QUALITY ASSURANCE:

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

10. TEST SYSTEM/CROP:

Clover (seed crop) - Use a commercial variety of established clover. Report: variety, source, lot number, date received, and other descriptive information if available.

Field 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 23 and 11.4 for requirements to ensure proper differentiation of multiple trials.

11. TEST SYSTEM DESIGN and STATISTICAL METHOD:

11.1 Each test site will consist of one untreated and one treated plot **OR** two treated plots. Two treated plots may be used for the collection of forage and hay samples. If only one treated plot is being employed for both forage and hay samples, be sure to flag/mark the area where the forage sample was taken (if it is not visibly obvious).

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

Field trial 13092.24-OR255 will provide samples for a decline trial (multiple sampling dates for the treated plot). The treated plot(s) must be large enough to provide enough samples on each sampling date to meet sample size requirements.

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

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

11.4 This section applies when a Field Research Director (FRD) has been assigned more than one trial in this study, or when two or more trials assigned to different FRDs are located within 18.6 miles (30 km) of each other.

An independently prepared tank-mix must be used in each trial.

Also, choose at least one option from below:

Option	Description
A	Trial sites must be separated by at least 30 km (18.6 miles) [measured as straight line distance]
B	Planting date (for annual crops) or first application date in each trial is 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.

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:

Prepare or select a test site that has been maintained following good local agricultural practices for the production of clover (seed crop) 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 fertilizer history of a minimum of 1 year and preferably 3 years.

13. TEST/CONTROL SUBSTANCE:

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

Use the Solicam DF herbicide formulation (78.6% active ingredient, 0.786 lb ai/lb product) of norflurazon (EPA Reg No. 61842-41, CAS# 27314-13-2) that has been characterized to meet GLP standards.

-Upon receipt, document the lot/batch number, condition, quantity received and if GLP characterized. 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.

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

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

Study completion can be confirmed by contacting the Study Director or the Regional Field Coordinator, or by searching the IR-4 web site; click on "Food Crops" and under the "IR-4 Food Crops Database" click on the "Test Substance Container Disposal Approval" link. URL: https://ir4app.cals.ncsu.edu/ir4foodpub/SubstanceDispoSch

Alternatively, some registrants will archive the test substance containers. If test substance containers are shipped to another location, the shipment must be conducted in accordance with local, state, and Federal regulations. See shipping documents for directions for return of the test substance; if none are given, contact the registrant representative: TKI NovaSource, Sandra Alcaraz, (602) 346-1937, email: salcaraz@tkinet.com, and Bob West, (480) 319-1223, email: Bob.West@tkinet.com.

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

An optional tracking form that may be used to confirm that the correct test substance has been received (with the correct label and Certificate of Analysis) is available at: https://ir4works.org/resources/frd-resources/.

14. TEST SUBSTANCE APPLICATION:

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

- Use application equipment that will provide uniform application of the test substance and result in adequate canopy penetration and coverage.
- The test substance, if applied in a mixture, must be applied to the test system within 30 minutes of mixing, otherwise the mixture must be agitated just prior to making the application to ensure that it is well mixed. (The additional agitation should be documented in Part 6G of the Field Data Book.) The mixture must always be applied to the test system within 2 hours of mixing.
- Each field trial requires a unique spray mixture. Do not use the spray mixture from one field trial on another field trial.

For soil applications of any type, see IR-4 Advisory #2004-02 for clarification of terminology: https://ir4.cals.ncsu.edu/other/Advisories/Final2004-02on10Dec04.pdf

Contact the Study Director if guidance is needed.

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

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

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

Discharge/Output Calibrations:

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

YES: A full calibration is required just prior to the first application (allowable the day before the application, but calibration on the day of use is preferred). A single, full calibration may be used for multiple trials in the same study or multiple studies if the following conditions are met:

1. The first application in each trial is the day of the calibration or the following day.
2. Application parameters and equipment components remain the same for each of the trials.
3. A recheck is run in each of the trials after the first.

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

Recheck is required when:

1. The equipment has been moved from the location where the most recent full calibration or recheck has occurred. (A sprayer that has been calibrated or rechecked at a farm or research station and then used to make an application somewhere else on that same farm or research station is *not* considered to have been "moved".)
2. The equipment has been cleaned.

3. Nozzles are removed and placed back on.
4. CO_2 tank has been changed.

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

Full output calibration is required if:

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

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

Speed Calibrations:

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

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

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

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

Full speed calibration is required when:

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

Speed recheck is required when:

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

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

14.3 Actual Application Rate: Record actual application pass-times in the Field Data Book and verify the accuracy of the application against the protocol rate. **The application is considered acceptable if the accuracy is within ±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**
01	Untreated	Not Applicable	Not Applicable	Not Applicable	Not Applicable
02	Norflurazon	1.25 lb ai/acre	721 grams/acre	Broadcast	≥10 GPA

*The nominal concentration of the formulated test substance will be used in calculating application rates (see Section 13 for the nominal concentration).
**GPA=gallons per acre

If it appears that phytotoxicity has resulted from applications made in this trial, contact the Study Director. If possible, take one or more photographs and send them to the Study Director via email to facilitate the evaluation of crop/ test substance effects.

All trials: Make one (1) broadcast application before dormancy breaks (when a minimum of 2-3 trifoliates are present).

A minimum of ½ - 1 inch of rainfall or irrigation within 24 hours of application is necessary to incorporate the test substance into the soil.

In all field trials conducted in the state of California, phytotoxicity data must be collected at 7-14 days after each application using a 0-4 scale and entered into Field Data Book 6P (available on the IR-4 website). If an application interval is less than 7 days, then the assessment may be done at the next application date. If the crop is harvested within 14 days of the last application, then the assessment should be made on the day of harvest. If a rating of 1 or higher is given to a plot, then a follow-up rating is needed 7-14 days after that, even if there is no additional test substance application in the interim, unless this rating is given to the crop at harvest.

16. SUPPLEMENTAL CROP TREATMENTS:

Protect the integrity of the field trial by managing pests that may cause significant damage to the test crop. Use only maintenance pesticides that have been registered on this commodity by EPA or the corresponding agency in the country in which the trial is located. Apply according to labeled directions. Make identical applications to the untreated and treated plots. In a field trial with multiple sample collection dates for the treated plot, maintenance applications may be made on that treated plot that are not made on the untreated plot or other plots from which sample collection has been completed.

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

17. RESIDUE SAMPLE COLLECTION:

All trials except decline trial 13092.24-OR255: Collect two samples from each plot. Each sample should be representative of the entire plot (except plot ends).

Forage Samples:
Harvest and sample forage from at least 12 separate areas of each plot. Clover should be at the 4-8 inch to prebloom stage, at approximately 25-35% dry matter (percent dry matter may be estimated). Begin with the untreated plot first, and then sample the treated plot, or use separate personnel for each plot. Each sample should weigh a minimum of 2 lb (but preferably not more than 4 lb). Each sample should be collected during a separate run through the entire plot. If hay samples will be collected from the same plot as forage samples, mark the areas where the forage samples have been collected so that hay samples are not taken from regrowth in these same areas.

Hay Samples:
When the clover is in the early to full bloom stage, starting with the untreated plot or utilizing separate personnel for the treated and untreated plots, harvest the hay in each plot. Allow the hay to dry in the field to a moisture content of approximately 10-20% (80-90% dry matter). (Percent dry matter may be estimated.) If rainy weather is expected, the hay samples may be removed to a sheltered area for drying. (Do not use forced hot air to accelerate drying.) Document all drying procedures, and whether or not the samples have been moved to a sheltered area. When the hay has dried, collect samples from the untreated plot first, followed by the treated plot. Hay samples should weigh a minimum of 1 lb (but preferably not more than 3 lb). Determine (or estimate) and report the moisture content of the hay upon sampling (i.e. placing the samples in the sample bags).

Decline trial 13092.24-OR255 only: Collect additional treated forage samples as described above at 10 (±1) days before the first 4-8" to prebloom stage samples are collected, 5 (±1) days before 4-8" to prebloom stage, 5 (±1) days after 4-8" to prebloom stage, and 10 (±1) days after 4-8" to prebloom stage.

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

Place all samples in plastic-lined cloth bags. Bags may be obtained from the Regional Field Coordinator (Section 23). Identify each sample bag** with correct Field ID number, Test Substance (chemical name listed in Section 15), complete sample ID (see Section 18) and harvest/sampling dates. After residue sample collection, store samples in a freezer. If the samples cannot be placed into a freezer within one hour, use an appropriate method of cooling and temperature-monitoring samples in order to maintain integrity.

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

18. FIELD RESIDUE SAMPLE INVENTORY:

18.1 All Trials Except Decline Trial 13092.24-OR255:

SAMPLE ID	TRT#	TREATMENT	CROP STAGE AT HARVEST	MINIMUM SAMPLE SIZE	CROP FRACTION
FA	01	Untreated	NA	2 lb.	Forage
FB	01	Untreated	NA	2 lb.	Forage
FC	02	Norflurazon	Between 4-8" to prebloom stage	2 lb.	Forage
FD	02	Norflurazon	Between 4-8" to prebloom stage	2 lb.	Forage
HA	01	Untreated	NA	1 lb.	Hay
HB	01	Untreated	NA	1 lb.	Hay
HC	02	Norflurazon	Early to full bloom stage	1 lb.	Hay
HD	02	Norflurazon	Early to full bloom stage	1 lb.	Hay

18.2 Decline trial 13092.24-OR255:

SAMPLE ID*	TRT#	TREATMENT	CROP STAGE AT HARVEST	MINIMUM SAMPLE SIZE	CROP FRACTION
FA	01	Untreated	NA	2 lb.	Forage
FB	01	Untreated	NA	2 lb.	Forage
FE	02	Norflurazon	10 (±1) days before the 4-8" to prebloom stage	2 lb.	Forage
FF	02	Norflurazon	10 (±1) days before the 4-8" to prebloom stage	2 lb.	Forage
FG	02	Norflurazon	5 (±1) days before the 4-8" to prebloom stage	2 lb.	Forage
FH	02	Norflurazon	5 (±1) days before the 4-8" to prebloom stage	2 lb.	Forage
FC	02	Norflurazon	Between 4-8" to prebloom stage	2 lb.	Forage
FD	02	Norflurazon	Between 4-8" to prebloom stage	2 lb.	Forage
FI	02	Norflurazon	5 (±1) days after the 4-8" to prebloom stage	2 lb.	Forage
FJ	02	Norflurazon	5 (±1) days after the 4-8" to prebloom stage	2 lb.	Forage
FK	02	Norflurazon	10 (±1) days after the 4-8" to prebloom stage	2 lb.	Forage
FL	02	Norflurazon	10 (±1) days after the 4-8" to prebloom stage	2 lb.	Forage
HA	01	Untreated	NA	1 lb.	Hay
HB	01	Untreated	NA	1 lb.	Hay
HC	02	Norflurazon	Early to full bloom stage	1 lb.	Hay
HD	02	Norflurazon	Early to full bloom stage	1 lb.	Hay

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

19. RESIDUE SAMPLE HANDLING AND SHIPMENT:

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

For pre-shipment 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. If the analytical laboratory is close enough to the field site to permit delivery of the samples by field personnel on the day of sampling, then pre-shipment frozen storage is not required.

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.

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

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

Insert Field Data Book Part 8B and Part 8C (Sample Arrival Check Sheet) into each box or container used to ship sample bags. This documentation is needed even when field personnel transport the samples to the analytical laboratory.

For analysis, send samples to: TBD

20. FIELD DOCUMENTATION AND RECORD KEEPING:

All operations, data and observations appropriate to this study should be **recorded directly and promptly into the IR-4 Field Data Book, eFDB** or Electronic Field Data Book and eFDB paper raw data. The term "Field Data Book" or eFDB refers collectively to the Electronic Field Data Book and eFDB paper raw data.

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

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

20.11- Residue sample shipping information

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

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

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

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

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

20.17–Data collected during sample drying, including a description of the drying method and the length of drying time

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

Additional requirements associated with using an eFDB are:

1. The Field Research Director(s) and other trial personnel who enter electronic raw data will have documented eFDB training from an IR-4 eStudy Administrator prior to entering electronic raw data.
2. The field users will follow the IR-4 Headquarters' SOP 5.8 for use of the electronic field data books. Unless Test Site SOPs have been updated to pertain to the eFDB, portions that pertain to the paper FDB will apply to the eFDB and paper raw data, unless otherwise described in a protocol or SOP change.
3. The individual field Test Site SOPs do not need to address the eFDB to be in compliance with GLP, because this protocol provides the required SOP elements for eFDB use.
4. The eFDB program has been validated by IR-4 HQ and iAdvantage, which has demonstrated acceptable installation, performance, and operation for online and offline GLP raw data use.
5. Test Site user(s) must verify the Windows or iPad Mobile Edition software is performing appropriately on their device prior to using the device to enter data offline and for any of these reasons:
   a. Before first use of the offline eFDB software for a new field season
   b. After any significant operating system software update or device repair is performed
   c. After a new version of the Mobile Edition is released
6. Verification is performed according to the following procedures:

Open the Windows or iPad Mobile Edition and Login to the user account

a. Refresh the notebook list after selecting to check the Verification Study Notebook for the test site of the FRD. An eStudy Administrator will provide the Verification Study Notebook.

b. Select to Move Off Line the verification form for Part 2. Personnel.

c. Enter your name, initials, and username to the form and save the entries in the mobile edition.

d. Select to return the Part 2. Personnel form online.

e. Deselect the checkbox from the Verification Study Notebook and select Refresh to remove the notebook from the local list

f. Open the website and login to access the online eFDB for the Verification Study Notebook

g. Open the Part 2. Personnel form

h. Use a screen shot, snipping tool, or print screen to generate the file showing that the offline entries were populated in the online form.

i. Print this file and place it in Facility Records or similar for the eFDB device.

j. Make an entry into the eFDB Device maintenance log denoting that verification was performed, when performed, by whom, using what SOP or process, and where the location of the screen shot raw data is retained.

7. The Field Research Director is responsible for ensuring that eFDB device(s) are adequately functioning, suitably located for use and safe storage, and that there is adequate control of access to the device, particularly when forms are checked out on the device in the Mobile Edition.

8. The Field Research Director is responsible for a timely upload of offline (checked-out) eFDB forms.

9. The Field Research Director is responsible for maintaining an equipment maintenance log that documents any verification testing, and any significant modification, update, or repair to the device or software. The log will include:

   a. Device maker and model name

   b. Device identifying code (serial number, equipment ID, or similar)

   c. eFDB software version number

   d. Dates of maintenance, testing, and/or standardized operations. Who performed it, whether it was routine or the result of a malfunction, and if so, the nature of the defect, how and when discovered, and any remedial action taken to resolve.

10. Cleaning of the device should be performed by user(s) as needed and is not required to be documented on the equipment maintenance log.

11. Any paper raw data generated during the course of this study must be adequately retained, with secure access, and protected from deterioration. Trial specific paper raw data must be transcribed into the eFDB forms (unless explicitly not required) in a timely manner and scanned or otherwise uploaded into the documents section of the eFDB. Documents that have been uploaded are considered electronic raw data and must not be deleted from the eFDB.

12. A paper raw data notebook is provided in the attachments section of the eFDB, which is to be printed and used to collect and retain trial specific paper raw data. The instructions in that paper notebook will be followed.

13. Non-trial specific, facility file type, raw data may be provided as a scan or uploaded document only. The document must be archived appropriately and the location of the original must be provided.

14. Any form of data or documentation necessary for reconstruction of the trial that cannot be captured in the eFDB forms should be added to the Notes section or uploaded in the Document section.

15. The Website eFDB and the Mobile Edition display and recording of the eFDB entries have built in data field settings for certain numbers in the iAdvantage Fixed Forms 11,12, and 14. These user entered or calculated values are required to contain a specific number of digits displayed after the decimal point. The actual values entered by the user are changed by the system to append trailing zeros after the decimal. (i.e. an entry of 100 seconds is changed to 100.00 seconds). This does not change the absolute value of the entry, but ascribes an artificial level of precision for the entry, which should be considered superficial. This setting is to provide a consistent display of values and for the program to conduct calculations in the background on those values.

16. Any error, problem, concern, question, or comment regarding the eFDB should be provided to one of the eStudy Administrators. If they are not available, contact the appropriate RFC provided in Section 23. Section 24 provides the contact information for who to contact at IR-4 Headquarters, if the RFC is not available. Communications which apply to a specific trial in this study will be included in the study raw data.

eStudy Administrator: Philip Moore, IR-4 Project Headquarters, North Carolina State University, 1730 Varsity Drive, Venture IV, Suite 210, Raleigh, NC 27606, (615) 426-6175, E-mail: pmoore@ncsu.edu
eStudy Administrator: James Byrtus, IR-4 Project Headquarters, North Carolina State University, 1730 Varsity Drive, Venture IV, Suite 210, Raleigh, NC 27606, (919) 515-3017, E-mail: jpbyrtus@ncsu.edu

21. PROTOCOL/SOP MODIFICATIONS - FIELD RESEARCH:

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

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

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

22. FIELD RESEARCH REPORT/ARCHIVING:

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

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

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

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

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

Field Research Director	Field ID NO.	RFC	Test Crop
Dani Lightle, Oregon State University-FRC, North Williamette Research & Extension Center, 15210 NE Miley Rd, Aurora, OR 97002; 503-694-9790, e-mail: Danielle.lightle@oregonstate.edu	13092.24-OR256	WSR	Clover (Seed Crop)
Dani Lightle, Oregon State University-FRC, North Williamette Research & Extension Center, 15210 NE Miley Rd, Aurora, OR 97002; 503-694-9790, e-mail: Danielle.lightle@oregonstate.edu	13092.24-OR255 (decline)	WSR	Clover (Seed Crop)
Wilson Peng, Washington State University, IAREC, 24106 N. Bunn Road, Prosser, WA 99350; 509-786-9291; Cell#: 626-756-0690; e-mail: wpeng@wsu.edu	13092.24-WA316	WSR	Clover (Seed Crop)
Wilson Peng, Washington State University, IAREC, 24106 N. Bunn Road, Prosser, WA 99350; 509-786-9291; Cell#: 626-756-0690; e-mail: wpeng@wsu.edu	13092.24-WA315	WSR	Clover (Seed Crop)

RFC = Regional/ARS Field Coordinator

Location:

ARS: Dr. Alvin Simmons, USDA-ARS, US Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414; Tel: (843) 402-5307; e-mail: Alvin.simmons@usda.gov

NER: Ms. Marylee Ross, University of Maryland, LESREC, 27664 Nanticoke Rd., Salisbury, MD 21801; Tel: (410) 742-8788 x 310; e-mail: mross@umd.edu

NCR: Nicole Soldan,IR-4 North Central Research Center, Michigan State University, 1066 Bogue St. Plant and Soil Sciences Building – Rm A440, Michigan State University, East Lansing, MI 48824; Cell: 517 712-8441; Email: schroe65@msu.edu;

SOR: Until Feb 29, 2024, Dr. Janine Spies, Univ of Florida, 1642 SW 23rd Drive, Bldg 833, PO Box 110720, Gainesville, FL 32611-0720, Ph: 352-294-3991; e-mail: jrazze@ufl.edu

Effective March 1, 2024, Kristen Searer-Jones, Univ of Florida, 1642 SW 23rd Drive, Bldg 833, PO Box 110720, Gainesville, FL 32611-0720. Ph: 352-294-3979; e-mail: k.searerjones@ufl.edu

WSR: Dr. Kari Arnold, Regional Field Coordinator, Western Region IR-4 Project, 4218 Meyer Hall, University of California-Davis, Davis, CA 95616 (530) 752-7634; Cell# (530) 574-9181; e-mail: klarnold@ucdavis.edu

CANADA: Christine Gagnon, (Mailing) Agriculture & Agri-Food Canada, Pest Management Centre, Building 57, CEF, 960 Carling Avenue, Ottawa, ON Canada K1A 0C6, Cell: (343) 550-1047; e-mail: christine.gagnon2@agr.gc.ca (Shipping): GLP Admin, Agriculture & Agri-Food Canada, Pest Management Centre, Building 57, CEF, 960 Carling Avenue, Ottawa, ON Canada K1A 0C6; Tel: 613-759-6625; e-mail: aafc.glpadmin-adminbpl.aac@agr.gc.ca

24. LABORATORY PERSONNEL/ID NO.: **LAB ID NO.: 24-TBD**

LABORATORY RESEARCH DIRECTOR/TESTING LABORATORY:

TBD

25. LABORATORY SAMPLE INVENTORY:

Treated and untreated samples of clover (seed crop) will be received from each of the trial sites in Section 23.

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

26. LABORATORY SAMPLE IDENTIFICATION:

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

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

27. LABORATORY SAMPLE STORAGE/PREPARATION:

Store samples in a limited access area at temperatures generally less than -18 °C (0 °F), allowing for normal variations of less than 24 hours' duration due to freezer cycling, sample movement, etc., 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 ≤ 4°C for no longer than 14 days before analysis.

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

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

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

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

28. LABORATORY REFERENCE SUBSTANCE:

Obtain the laboratory reference substance(s), norflurazon and desmethyl norflurazon, from the Registrant. Contact TKI NovaSource, Sandra Alcaraz, (602) 346-1937, email: salcaraz@tkinet.com, and Bob West, (480) 319-1223, email: Bob.West@tkinet.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:

PR Notice 88-5 Enforcement Method Validation for Norflurazon and Desmethyl Norflurazon in Plant Material, Pan-Ag Study No.: PAL-AL-91-120; S. Schimelfining, 1991.

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 norflurazon and desmethyl norflurazon at a minimum of 3 concentration levels each, lowest level of method validation 0.02 ppm or lower), 0.2 ppm, and 2 ppm.

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

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

Document the exact procedures for sample analysis.

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

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

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

SAMPLE ANALYSIS:

Samples will be analyzed for the total and/or combined residues of norflurazon and desmethyl norflurazon 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:

Storage stability samples shall not be fortified until after the working method has been successfully validated. Once the method is validated, a minimum of nine (9) subsamples of a control from each crop fraction shall be fortified with norflurazon and desmethyl norflurazon at 0.2 ppm each. Three fortified subsamples must be analyzed on the day of fortification to document the residues in the 0-day storage stability sample. If it is anticipated that the lab will not be able to analyze the study samples within a year after receipt of the last field sample from the study, consult with the study director prior to spiking the storage stability samples to determine whether more than nine subsamples will be spiked for storage stability analyses. For storage of samples longer than a year, analyses of additional intervals may be required.

Sufficient storage stability data covering the storage of samples for **39 months** has been reported by the registrant. Storage stability analyses for this study will be run only if sample storage exceeds the aforementioned storage period(s) reported by the registrant.

Contact the Study Director to determine if Storage Stability Samples need to be analyzed.

If storage stability analyses will be required, discuss with the study director what storage stability intervals will be analyzed. Only if directed by the Study Director, three samples of each analyte and crop fraction will be analyzed at each analysis interval and after the appropriate storage period. The analysis of the final interval of storage stability samples may be conducted following a storage period equal to or greater than 100% of the longest storage period of the field –treated samples from collection in the field/processing facility until their analysis. The remaining samples will be retained for long-term storage.

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

STATISTICAL METHOD(S):

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

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

30. DISPOSITION OF SAMPLES:

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

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

Sample extracts can be disposed of after data analysis.

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

31. LABORATORY PROTOCOL/SOP MODIFICATIONS - LABORATORY RESEARCH:

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

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

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

32. LABORATORY DOCUMENTATION AND RECORD KEEPING:

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

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

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

32.02 - Analytical standard(s) storage conditions

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

32.04 - Sample storage conditions and locations

32.05 - Calculation work sheets

32.06 - All chromatograms, including those that are not reported

32.07 - Chain of custody records

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

32.09 - Name of personnel conducting specific research functions

32.10 - Sample analysis worksheets

32.11- Storage stability fortification records

32.12 - Concurrent recovery fortification records

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

33. LABORATORY RESEARCH REPORT:

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

33.01 - Applicable method validation data

33.02 - Applicable storage stability data

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

33.04 - Complete copy of the analytical Working Method

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

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

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

33.08 - Clearly presented example calculations or statistical evaluations

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

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

34. LABORATORY ARCHIVES:

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

For studies assigned to any other analytical laboratory: When the final analytical summary report is completed the analytical report and all original raw data will be sent to IR-4 Project Headquarters, ***North Carolina State University, 1730 Varsity Drive, Venture IV, Suite 210, Raleigh, NC 27606*** (when an original document cannot be provided a "true copy" will be provided). All original raw data shall be secured in the archives of IR-4 Headquarters. A "true copy" of the raw data and the final analytical report shall be secured in the archives of the Laboratory Research Director/Testing Facility.

IR-4
FIELD DATA BOOK

TITLE: NORFLURAZON MAGNITUDE OF THE RESIDUE ON CLOVER (SEED CROP)

PR# 13092

SPONSOR

IR-4 Project Headquarters
North Carolina State University
1730 Varsity Dr.
Venture IV, Suite 210
Raleigh, NC 27606
(919) 515-1552

STUDY DIRECTOR

Christina Dineen
(919) 515-6596
cdineen2@ncsu.edu