GLP Analytical Laboratory Procedures and Report

Dr. Pim Larsson-Kovach
IR-4 Analytical Laboratory Director
Cornell University
Geneva, NY USA
The Scope of GLP Laboratory Operations

- Sample Receipt, Storage, and Processing
- Reference Standards and Solutions
- Working Method, Modifications, and Method Validation
- Sample Analysis and Storage Stability
- Analytical Summary Report
- ( Provision of Quality Assurance )
Sample Receipt, Custody, and Storage

- Frozen samples are shipped to the Laboratory by freezer trucking or overnight express in containers with dry ice present.
- Sample conditions are checked upon receipt.
- Samples identified with shipping form/protocol and logged in.
- Unique lab numbers are cross referenced to field sample numbers.
- Samples are stored frozen at all times in temperature controlled and monitored freezers.
### 18. FIELD RESIDUE SAMPLE INVENTORY:

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>TRT#</th>
<th>TREATMENT</th>
<th>DAYS AFTER LAST APPLIC.</th>
<th>MINIMUM NO. OF SAMPLE</th>
<th>CROP FRACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>01</td>
<td>Untreated</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
<tr>
<td>B</td>
<td>01</td>
<td>Untreated</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
<tr>
<td>C</td>
<td>02</td>
<td>FLUMIOXAZIN</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
<tr>
<td>D</td>
<td>02</td>
<td>FLUMIOXAZIN</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
<tr>
<td>E</td>
<td>03</td>
<td>FLUMIOXAZIN</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
<tr>
<td>F</td>
<td>03</td>
<td>FLUMIOXAZIN</td>
<td>NA</td>
<td>12 heads</td>
<td>Head with Wrapper Leaves</td>
</tr>
</tbody>
</table>
### Field ID No:
0519.06-NY08
Fluorescent/Chlorophyll
Bulldozer

### IR-4 Field Data Book

#### Part 8. Residue Sample Shipping

**B. Residue Sample Chain of Custody Form**

**Instructions:** Complete this form for each sample shipment. Place a true copy within each shipping container and mail this true copy to the study director AND to your Regional Field Coordinator. Retain the original in the field data book.

**Test Substance:** Flumioxazin

**Crop:** Cabbage

**Field Research Director:** Robin Delinder

**Phone:** 607-255-7890

**Fax:** 607-255-0599

**Trial Location:** Frearville, NY (Cornell U.)

**Number of Boxes Shipped:** 3

**Total Number of Samples Shipped:** 5 samples (5 samples)

**Destination:** NYSACS, Geneva, NY

**Carrier:** ACDS

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Treatment/Rate</th>
<th>No. of Applics</th>
<th>Date of Last Application</th>
<th>Date Harvested</th>
<th>Date Sampled</th>
<th>Crop/Fraction</th>
<th>LAB ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>T01 / NA</td>
<td>NA</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>leaf, whole</td>
<td>06377</td>
</tr>
<tr>
<td>B</td>
<td>T01 / NA</td>
<td>NA</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>leaf, whole</td>
<td>06378</td>
</tr>
<tr>
<td>C</td>
<td>T02 / 0.047</td>
<td>0.047</td>
<td>5-22-06</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>hand, whole</td>
<td>06379</td>
</tr>
<tr>
<td>D</td>
<td>T02 / 0.047</td>
<td>0.047</td>
<td>5-22-06</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>hand, whole</td>
<td>06380</td>
</tr>
<tr>
<td>E</td>
<td>T03 / 0.047</td>
<td>0.047</td>
<td>5-22-06</td>
<td>8-24-06</td>
<td>8-24-06</td>
<td>hand, whole</td>
<td>06381</td>
</tr>
</tbody>
</table>

**F** - This sample was not sent today.

**No Harvest Material to complete this sample.

**Received:** 8-25-06

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**Part 8 Page Of**

**Date:** 8-25-06

**Trial Year 2006**

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**Complete if Appropriate:** "This is a true copy of the original."  
**The original is in IR-4 Field Data Book No.** ___________  
**Initials** ___________  
**Date** ___________
IR-4 NATIONAL PESTICIDE CLEARANCE RESEARCH PROGRAM
SAMPLE ARRIVAL CHECK SHEET
IR-4 Northeast Regional Laboratory
Cornell University
New York State Agricultural Experiment Station
Geneva, New York 14456

INSTRUCTIONS: This is notification of samples received by the IR-4 Northeast Regional Laboratory. Please file this exact copy with the appropriate project.

FR. No.: 09519
Field ID No.: 09519.06-NY08
Lab ID No.: 09519.06-NYR10
CHEMICAL: Flumioxazin
COMMODITY: Cabbage
COOPERATOR: Robin Bellinger
SHIPPER: [X] ACDI [ ] FedEx [ ] DHL [ ] OTHER:
SHIPPING REFERENCE NO.: 101366
# OF BOXES: 3
DATE RECEIVED: 8/25/06
BY: Pim Kovach

A. CONDITION OF SAMPLES:
Form Of Samples As Received:
[x] Frozen
[ ] Thawed
[ ] Dry Ice Present
[ ] Fresh, Never Frozen
Residue Samples Shipping & Identification Form Enclosed
All Samples Present In Agreement with Shipping Form
Sample Bags Intact
Sample Bags Broken or Open and Contents Mixed

Head with wrapper leaves

B. SAMPLE SHIPPING FORM IN AGREEMENT WITH PROTOCOL/AMENDMENT(S):
Protocol:
Field Treatment: Untreated/Treated
Number of Applications: 1
Crop Fraction: Head with wrapper leaves
PHI(s): NA
Harvest Date(s): 8/24/06
No. of samples: 5
A4_Rceived:

C. NOTIFICATION OF ARRIVAL:
[x] Field Research Director: Bellinger
[x] Regional Field Research Coordinator: Lurie
[x] Study Director: Arenovici

D. SAMPLE LOG:
[x] Flag #: 060053
[x] Lab Numbers Assigned: 06377, 06381
[x] Project Listed on Master Schedule
Freezer Number: G-10
Temperature: -21.7°C

E. COMMENTS: * Sample ID # F was not harvested due to crop damage.

Logged In By: Jane DeCann

Rev 3/03 JWD - NYSAES 9/10/06
<table>
<thead>
<tr>
<th>Lab ID #</th>
<th>Sample ID</th>
<th>TRT #</th>
<th>Treatment</th>
<th># of Applic.</th>
<th>Date of Last Applic.</th>
<th>Date Harvested</th>
<th>Date Sampled</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>06377</td>
<td>A</td>
<td>01</td>
<td>Untreated</td>
<td>0</td>
<td>NA</td>
<td>8/24/06</td>
<td>8/24/06</td>
<td>Head with wrapper leaves</td>
</tr>
<tr>
<td>06378</td>
<td>B</td>
<td>01</td>
<td>Untreated</td>
<td>0</td>
<td>NA</td>
<td>8/24/06</td>
<td>8/24/06</td>
<td>Head with wrapper leaves</td>
</tr>
<tr>
<td>06379</td>
<td>C</td>
<td>02</td>
<td>Treated</td>
<td>1</td>
<td>5/22/06</td>
<td>8/24/06</td>
<td>8/24/06</td>
<td>Head with wrapper leaves</td>
</tr>
<tr>
<td>06380</td>
<td>D</td>
<td>02</td>
<td>Treated</td>
<td>1</td>
<td>5/22/06</td>
<td>8/24/06</td>
<td>8/24/06</td>
<td>Head with wrapper leaves</td>
</tr>
<tr>
<td>06381</td>
<td>E</td>
<td>03</td>
<td>Treated</td>
<td>1</td>
<td>5/22/06</td>
<td>8/24/06</td>
<td>8/24/06</td>
<td>Head with wrapper leaves</td>
</tr>
</tbody>
</table>

Received: 5 Samples on 8/25/06

Flag # 060053
Sample Processing

- The total sample has to be processed (ground, cut, chopped, etc.) and thoroughly mixed.
- Sample integrity is maintained by grinding with dry ice.
Sample Processing, continued

- Untreated controls are ground before treated samples to prevent contamination.
Sample Processing, continued

- Portions of each processed sample are stored frozen in glass jars (sometimes plastic/nalgene) at all times, each labeled either analyst or archive.
Sample Processing, continued

- Storage stability samples are prepared at this time, if required in study protocol, in order to show that samples are stable under the conditions stored (not a guideline storage stability study).

- When an analytical study is initiated, the field samples are signed out to a designated analyst and brought to a laboratory freezer. Copies of the Sample Summary Sheets are kept in a notebook for the analyst. Sample numbers are identified and each sample move has to be recorded in a log book.
Laboratory Research Director (LRD) or other designated personnel contacts (by email, fax, or telephone) the Chemical Company (registrant) representative cited in the study protocol to receive appropriate standard(s) and documentation.

Reference standards should be shipped with Certificate of Analysis (COA) which must include lot number, purity, storage conditions, characterization, and expiration dates.
Reference standard must be characterized by the registrant under GLP.

Reference standards are logged into a Primary Reference Standard Book and a primary standard card is filled out.
Stock solutions and serial dilutions for use in the study are usually prepared according to the reference method cited in the protocol.
Reference Standards and Solutions, continued

- The % purity of the reference standard is taken into account when the stock solution concentration is calculated.
## IR-4

### Preparation of Calibration and Spiking Standard Solutions

**Solvent:** Acetone  **Lot #:** 13749  **Fisher ( ) or Other:** 

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Compound</th>
<th>Conc. (µg/ml)</th>
<th>Aliquot Vol (µL)</th>
<th>Final Vol (µL)</th>
<th>Final Conc. (µg/mL)</th>
<th>Mixed</th>
<th>Single</th>
<th>Date Prepared/ Initials</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>100</td>
<td>5.0</td>
<td>100</td>
<td>5.0</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>100</td>
<td>2.0</td>
<td>100</td>
<td>2.0</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>100</td>
<td>1.0</td>
<td>100</td>
<td>1.0</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>100</td>
<td>0.50</td>
<td>100</td>
<td>0.50</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>1.0</td>
<td>0.0</td>
<td>50</td>
<td>0.20</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>1.0</td>
<td>0.0</td>
<td>50</td>
<td>0.20</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
<tr>
<td>576</td>
<td>Flumioxazin</td>
<td>1.0</td>
<td>0.0</td>
<td>50</td>
<td>0.20</td>
<td></td>
<td></td>
<td>1/1/07</td>
<td>1/1/08</td>
</tr>
</tbody>
</table>

**COMPLETE IF APPROPRIATE:** "THIS IS A TRUE COPY OF THE ORIGINAL"

THE ORIGINAL IS IN IR-4 STUDY NO.       INITIALS       DATE
Reference Standards and Solutions, continued

- All containers of reagents and solutions must be labeled correctly including chemical name, concentration, solvent (when used), preparation, and expiration dates as well as storage conditions.

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>(AMBIENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONC</td>
<td></td>
</tr>
<tr>
<td>DATE</td>
<td>BY</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>(REFRIGERATE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONC</td>
<td></td>
</tr>
<tr>
<td>DATE</td>
<td>BY</td>
</tr>
</tbody>
</table>
Working Method

- The protocol includes an IR-4 method (registrant reference method) that mainly should be followed.
- Some method development is needed to verify the validity of the method for different matrices.
- “Minor Modifications” can be used but original extraction procedure (solvents) should be left the same.
- Sample weight and volumes may be changed proportional to the original method (e.g., fresh and dry matrices).
- Clean up steps can be modified or removed depending on matrix.
- Equipment can be exchanged as long as the same functions are carried out as in the reference method.
Working Methods, continued

- Instruments are either GC with EC, NP, or MS detection for volatile, thermally stable compounds or LC with UV, FL, or MS detection for nonvolatile and/or thermally labile compounds.
Working Method, continued

- Nowadays LC/MS or LC/MS/MS is more frequently used but many methods still include derivatization to more volatile compounds for GC analysis.
Changes from GC to LC method will require independent MV and is therefore not recommended.

All changes/modifications should be discussed with and approved by the Study Director (SD) prior to sample analysis.

Once the reference method is modified for a particular commodity it becomes the enforcement method for EPA.
Method Validation (MV)

- Usually three recovery spike concentrations in triplicates are required as per protocol.
- Lowest level of method validation (LLMV) and two higher concentrations.
Method Validation, continued

- The SD is informed of the recovery results and the working method.
- Recoveries of 70 - 120% are accepted.
- If outside this range, SD approval or additional MV recovery spikes are required.
Sample Analysis

- Usually four samples (1 control + 2 treated + 1 concurrent recovery spike) are weighed, extracted, and cleaned up in one analytical set.
Sample Analysis, continued

- Samples weighed are labeled with the previously assigned unique lab number plus a lower case “a-z”.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Description</th>
<th>Sample Weight</th>
<th>Sample #</th>
<th>Conc (µg/mL)</th>
<th>Vol.</th>
<th>1. Aliquot Weight (µg)</th>
<th>2. Aliquot Weight (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>83293.1</td>
<td>Extracted</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>83732.3</td>
<td>Extracted</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>83743.1</td>
<td>Extracted</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>83751.2</td>
<td>Extracted</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Sample other comments...

Sample Preparation:
- Procedure: Residue Analysis of Fluorocabon by GC/Navrogo Phosphorus Detector
- Date/Initial: 8/15/07, BDL

- Clean-up
- Injection Date: 8/16/07

Extract Storage Refrigerator # 17b

Analysis: 8/16/07
Date: 8/16/07
Reviewed by: 8/16/07
Sample Analysis, continued

- In general two injections of each extracted sample and the average are reported in ppm.
Sample Analysis, continued

- During the course of residue sample analysis, adequate concurrent recovery spikes bracketing the actual residues have to be analyzed.

- If recoveries outside of 70 - 120%, set will first be re-injected and if still not satisfactory another set will be extracted and reanalyzed in agreement with the SD.
Sample Analysis, continued

- Controls with interference or contamination as well as field samples with unusual residues must be reported to the SD promptly.

- During MV as well as during field sample analysis instrument parameters and conditions should stay constant and are recorded in an instrument log book.
Before completion of the analytical phase of the study a minimum of six recovery spikes at the LLMV are required in order to calculate the LOD and LOQ.
Definitions

- **LLMV** is the lowest concentration level at which the method is validated for a particular matrix.
- **LOD** is the smallest amount of the analyte that can be reliably detected from the background for a particular matrix.
- **LOQ** is the smallest amount of the analyte that can be quantified with a certain degree of reliability within a particular matrix.
Storage Stability

- IR-4 does not perform guideline storage stability studies.
- One time analyses are carried out to show that samples are stable under the conditions stored.
- Storage stability analysis is performed after the longest interval between harvest and extraction of a field sample.

### IR-4 NATIONAL PESTICIDE CLEARANCE RESEARCH PROGRAM

#### RESIDUE DATA REPORTING FORM

**Storage Stability Results for Flumioxazin in Cabbage**

<table>
<thead>
<tr>
<th>PR No.: 09519</th>
<th>Lab ID No.: 09519:06-NYRI0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analyte:</strong></td>
<td>Flumioxazin</td>
</tr>
<tr>
<td><strong>Crop Fraction:</strong></td>
<td>Heads</td>
</tr>
<tr>
<td><strong>Parent Compound:</strong></td>
<td>Flumioxazin</td>
</tr>
<tr>
<td><strong>Date Fortified:</strong></td>
<td>6/28/06</td>
</tr>
<tr>
<td><strong>Stage added:</strong></td>
<td>to ground subsample</td>
</tr>
<tr>
<td><strong>Form added:</strong></td>
<td>Flumioxazin in Acetone</td>
</tr>
<tr>
<td><strong>Analyte:</strong></td>
<td>M. B. Sterling</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory Sample #</th>
<th>Fort Conc. (ppm Flumioxazin)</th>
<th>Number of Days Stored</th>
<th>Flumioxazin Conc. (ppm)</th>
<th>% Recovery</th>
<th>Report Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>06122-1I</td>
<td>0.20</td>
<td>243</td>
<td>0.24</td>
<td>120</td>
<td>109</td>
</tr>
<tr>
<td>06122-1H</td>
<td>0.20</td>
<td>243</td>
<td>0.24</td>
<td>120</td>
<td>109</td>
</tr>
<tr>
<td>06122-1II</td>
<td>0.20</td>
<td>243</td>
<td>0.21</td>
<td>105</td>
<td>109</td>
</tr>
</tbody>
</table>

**Concurrent Recoveries**

<table>
<thead>
<tr>
<th>Laboratory Sample #</th>
<th>Spiking Conc. (ppm Flumioxazin)</th>
<th>% Recovery</th>
<th>Average % Recovery</th>
<th>Report Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>06122-b</td>
<td>0.20</td>
<td>110</td>
<td>Average</td>
<td>109</td>
</tr>
</tbody>
</table>

Data for 2/26-3/7/07 recoveries
Daily Results

- Calibration curves, chromatograms, daily sheets, and daily result tables are submitted by the analyst to the LRD for scientific review upon completion of every analytical set.
Calibration Curves

Flumioxazin at exp. RT: 9.153
RSD: 0.99865
Residual Std. Dev.: 10.95261
Formula: y = mx + b
n: 175.36540
b: -6.69419
x: Amount (ug/ml)
y: Area
PR#: 09519  Flumioxazin
Matrix: Cabbage
Parent Compound: Flumioxazin

Lab ID#: 09519.06-NYR10
Sample Weight(g): 10
Final Volume (mL): 1.0
Multiplier: 0.10

Sample Name:
#06378-c Cabbage
Untreated Control

Injection Volume (uL): 5.0
Vial: 9
Sequence Line#: 10
Injection#: 1

Injection Date: 2/6/2007 9:13:08 PM
Report Created: 2/7/2007
Analyst: M.B. Sterling

Acquisition Method: FLUMICA.M

---

Retention Time | Compound Name | Area | Amount (ppm)
---------------|--------------|------|--------------
0.000          | Flumioxazin  | 0    | 0.0000      
**PR#: 09519 Flumioxazin**
**Matrix: Cabbage**
**Parent Compound: Flumioxazin**

**Lab ID#: 09519.06-NYRI0**
**Sample Weight(g): 10**
**Final Volume(mL): 1.0**
**Multiplier: 0.10**

**Sample Name:**
#06380-b Cabbage
Treated Sample

**Injection Volume(μL): 5.0**
**Vial: 11**
**Sequence Line#: 14**
**Injection#: 1**

---

**Injection Date:** 2/7/2007 12:01:59 AM
**Report Created:** 2/7/2007
**Acquisition Method:** FLUMICA.M

---

**Data Table:**

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Compound Name</th>
<th>Area</th>
<th>Amount (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>Flumioxazin</td>
<td>0</td>
<td>0.0000</td>
</tr>
</tbody>
</table>
PR#: 09519  Flumioxazin
Matrix: Cabbage
Parent Compound: Flumioxazin

Sample Name:
#06255-d Cabbage
Untreated Control+Recovery Spike
0.020ppm Flumioxazin

Injection Date:1/31/2007 3:16:35 AM
Report Created:1/31/2007
Acquisition Method:FLUMICA.M

Injection Volume(uL): 5.0
Vial 13
Sequence Line#:18
Injection#:1

Analyst: M.B. Sterling

Retention Time | Compound Name | Area | Amount (ppm)
--------------|---------------|------|-------------
9.199         | Flumioxazin   | 32   | 0.0276
Daily Results, continued

- A standard curve has to be run with every analytical set.
- Each analytical run has to start and end with a calibration standard.
- Calibration standards should bracket the expected residues and recovery concentrations.
- \( r^2 \) should be \( \geq 0.95 \)
- Results are shared with the SD on a regular basis.
Analytical Summary Report (ASR)

- When the sample analysis section of the study is completed the LRD prepares an ASR.
- The ASR should contain but not be limited to the following:
ASR, continued

- Title page
- GLP compliance statement (signed)
- QA statement (signed)
- Lab personnel
- Table of Content
- Location of raw data

- Reference standard inventory and history
- Sample inventory and history
- Summary (includes introduction, working method, analytical results of MV with LOD and LOQ, residue sample results, and storage stability results)
ASR, continued

- Summary tables of MV, field study, and storage stability
- Calibration standards and sample chromatograms
- Daily sample analysis sheets
- Reference standard GLP Certificate of Analysis
Thank you!