

---

**Ricerca, Inc.**

Study Title:

**ENVIRONMENTAL FATE OF Cry1F PROTEIN  
INCORPORATED INTO SOIL**

Data Requirements:  
Not Applicable

Study Completed on:  
December 21, 1998

Laboratory Project ID:  
7569-98-0080-AC

**SUBMITTED BY:**  
Diane Shanahan  
Product Registration Manager

Mycogen  
5501 Oberlin  
San Diego, CA 92121

**ON BEHALF OF THE APPLICANT:**

Mycogen  
5501 Oberlin Dr.  
San Diego, CA 92121

## STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in its study on the basis of its falling within the scope of FIFRA 10(d)(1)(A),(B),(C).

“We submit this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material to the EPA according to method and format requirements contained in PR Notice 86-5. We do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA”

Company:

Company Agent: *Diane Shanahan*

Diane Shanahan  
Product Registration Manager

Mycogen  
5501 Oberlin Drive  
San Diego, CA 92121


Date:

*9/2/99*

## COMPLIANCE STATEMENT

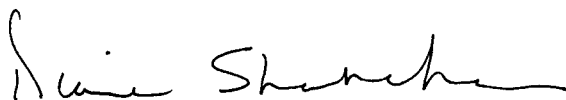
The work reported in this report "Environmental Fate of Cry1F Protein Incorporated into Soil", Ricerca Document No. 7569-98-0080-AC-001, was conducted and reported in compliance with the Good Laboratory Practice Standards set forth in Title 40, part 160 of the Code of Federal Regulations of the United States of America.

The stability, homogeneity and chemical characterization of the test and control substances, per 40CFR160.105, were not determined by Ricerca, Inc. and were the responsibility of the Sponsor.

  
\_\_\_\_\_  
**W. Ross Halliday, Ph.D., B.C.E.**  
**Study Director**  
**Ricerca, Inc.**

Dec 21, 1998  
**Date**

*The above statement is complete and unaltered.*

  
\_\_\_\_\_  
**Applicant**

9/2/99  
**Date**

**REPORT**

**Study Title**

**Environmental Fate of Cry1F Protein Incorporated into Soil**

**Ricerca, Inc. Document No. 7569-98-0080-AC-001**

**Author**

**W. R. Halliday, Ph.D.**

**Testing Facility**

**Ricerca, Inc.  
7528 Auburn Road  
P.O. Box 1000  
Painesville, OH 44077**

**Study Sponsor**

**Mycogen  
5501 Oberlin  
San Diego, CA 92121**

**Project Identification**

**7569**

**Sponsor's Representative**

**Diane Shanahan**

DISTRIBUTION

Document Number: 7569-98-0080-AC-001

Original

Ricerca Archivist

Copies

Mycogen/(1) bound, (2) unbound  
Biological Services Files/(3)  
W. R. Halliday/(4)

## APPROVALS

**Study Title:** Environmental Fate of Cry1F Protein Incorporated into Soil

**Document Number:** 7569-98-0080-AC-001

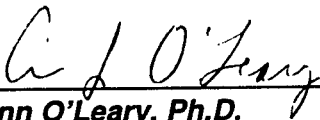
**Performing Laboratory:** Ricerca, Inc.  
7528 Auburn Road  
P.O. Box 1000  
Painesville, OH 44077-1000



\_\_\_\_\_  
**W. Ross Halliday, Ph.D., B.C.E.**  
**Senior Research Entomologist**  
**Biological Services**

*Dec. 21, 1998*

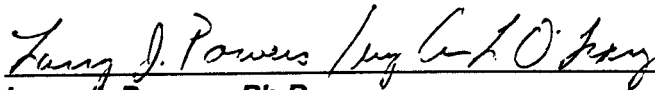
\_\_\_\_\_  
**Date**



\_\_\_\_\_  
**Ann O'Leary, Ph.D.**  
**Section Head**  
**Biological Services**

*Dec. 21, 1998*

\_\_\_\_\_  
**Date**



\_\_\_\_\_  
**Larry J. Powers, Ph.D.**  
**Director**  
**Analytical and Biological Services**

*Dec. 21, 1998*

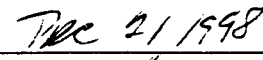
\_\_\_\_\_  
**Date**

## QUALITY ASSURANCE STATEMENT

The Ricerca, Inc. Quality Assurance Unit has performed inspections on the study, "Environmental Fate of Cry1F Protein Incorporated into Soil", Ricerca Document Number 7569-98-0080-AC-001. Listed below are the dates inspections were conducted by Quality Assurance and the dates those findings were reported to the Study Director and to management.

Date(s) of Inspection	Date(s) Reported to Ricerca Management	Date(s) Reported to Study Director
Sept. 4, 1998	Oct. 9, 1998	Sept. 9, 1998
Oct. 9, 1998	Dec. 4, 1998	Oct. 9, 1998
Nov. 3, 1998	Nov. 16, 1998	Nov. 4, 1998
Dec. 3,4,7,8, 1998	Dec. 16, 1998	Dec. 9, 1998

  
\_\_\_\_\_  
Quality Assurance Auditor

  
\_\_\_\_\_  
Date

## DEVIATIONS TO THE PROTOCOL:

- 1) Due to an oversight, the first two bioassay runs did not include fresh Cry1F or Dipel mixed into soil or by itself. Subsequent bioassays did include them.

This deviation is believed to have no effect on the outcome or integrity of the results of this study.

## TABLE OF CONTENTS

	<i>PAGE</i>
Title Page.....	1
Statement of No Confidentiality Claims .....	2
Distribution .....	5
Approvals .....	6
Quality Assurance Statement .....	7
Deviations to the Protocol:.....	8
Table of Contents .....	9
List of Tables.....	10
List of Appendices.....	11
Summary .....	12
Study Title .....	12
Study Number: 7569-98-0080-AC.....	12
Purpose/Objective .....	12
Schedule of Events .....	12
Study Sponsor .....	12
Testing Facility.....	12
Study Director .....	13
Materials and Methods .....	13
Test Procedure.....	13
Test Organisms.....	15
Water .....	15
Environmental Conditions .....	15
Weight Determinations .....	15
Statistical Analysis .....	15
Results .....	16
Discussion .....	18
Names of Key Individuals Involved in Study.....	19
Disposition of the Raw Data .....	19

**LIST OF TABLES**

	<i>Page</i>
Table 1: Results of individual bioassays (EC <sub>50</sub> values (ml)) of Cry1F samples incubated in the presence of Drummer Ap loam soil or alone over time.....	16
Table 2: Results of individual bioassays (EC <sub>50</sub> values (ml)) of DiPel™ samples incubated in the presence of Drummer Ap loam soil or alone over time. ....	17

## LIST OF APPENDICES

	<i>Page</i>
APPENDIX A Reduced, original copy of protocol and approval.....	20
APPENDIX B Reduced copy of characterization of test substance report.....	37
APPENDIX C Bioassay data.....	39
APPENDIX D SAS code used for generating ED <sub>50</sub> Values.....	50
APPENDIX E Results and methods for calculating DT <sub>50</sub> values for Cry1F and Dipel in soil.....	52
APPENDIX F Copy of GLP soil characterization report.....	54
APPENDIX G Moisture loss data.....	56
APPENDIX H Certified copies of temperature/relativity records for lo-cold freezer, incubation chamber (labeled C1), Forma scientific incubator and relevant pages from incubator logbook documenting pen malfunction.....	58

## **SUMMARY**

### **STUDY TITLE**

Environmental Fate of Cry1F Protein Incorporated into Soil

**STUDY NUMBER:** 7569-98-0080-AC

### **PURPOSE/OBJECTIVE**

To determine time dependent loss of activity of bacterially expressed Cry1F protein following incorporation into a typical maize-growing soil under laboratory conditions.

### **SCHEDULE OF EVENTS**

Study Initiation Date: August 27, 1998

Experimental Termination Date: November 19, 1998

Study Completion Date: December 21, 1998

### **STUDY SPONSOR**

Mycogen  
5501 Oberlin  
San Diego, CA 92121

### **TESTING FACILITY**

Ricerca, Inc.  
Analytical and Biological Services  
7528 Auburn Road  
P.O. Box 1000  
Painesville, OH 44077-1000

## STUDY DIRECTOR

W. R. Halliday, Ph.D., B.C.E.  
Senior Research Entomologist  
Ricerca, Inc.  
7528 Auburn Road  
Painesville, OH 44077

## MATERIALS AND METHODS

### TEST PROCEDURE

Protocol 7569-98-0080-AC-000 was followed (see Appendix A).

Purified Cry1F protein was incorporated into Drummer Loam soil at the rate of 500 µg per 4.05g soil (3.15 g dry weight) and placed in 50 ml plastic centrifuge tubes with a small (< 2.0 mm diameter) hole in the cap for gas exchange. According to the Sponsor, the bacterial Cry1F powder contained 11.4% Cry1F by weight.

Based on preliminary, range-finding studies, it was determined that 0.1 ml of a 1.85 mg/ml solution of Cry1F applied to soil would cause approximately 50% growth inhibition of *Heliothis virescens*. Therefore, for the Cry1F treatments, each experimental tube contained 500 µg of purified Cry1F powder representing 57 µg Cry1F. A certificate of analysis (provided by the Sponsor) is included with this final report.

Controls consisted of Cry1F powder incubated without soil. Because of the difficulty in weighing out exactly 25 mg of the purified Cry1F, aliquots weighing between 20 and 30 mg were placed in the control tubes. When used for bioassay, the volume of 0.2% agar was adjusted so that the final concentration of Cry1F was constant. DiPel™ was used as a standard and was tested alone and in soil. The test samples were incubated in a plexiglass chamber in the dark at 24 ± 2 °C with an aquarium pump-driven bubbler apparatus. The relative humidity was ca. 60-65% in the incubation chamber.

The tubes containing the samples were placed in the plexiglass chamber in a completely randomized block design. Four replicates were run simultaneously.

Soil (Drummer Ap horizon, 0-15 cm deep) was obtained from R. G. Darmody, University of Illinois at Urbana-Champaign. Agvise Laboratories (Northwood, ND) performed a GLP compliant analysis. Just prior to the experiment, soil moisture was brought to about 75% field capacity. Six tubes containing soil only were placed in the chamber to monitor water loss over the course of the study. These six tubes were weighed upon initiation of the study and at each time point. They were returned to the chamber after weighing.

The test duration was 28 days. Samples were removed for bioassay at 0, 3, 5, 7, 10, 14 and 28 days and immediately frozen at -70 °C until used in the bioassay.

The bioassay measured growth inhibition of *Heliothis virescens* larvae following exposure to a series of doses in a diet incorporation assay.

For the bioassay, samples were brought up to 20 ml with 0.2% agar solution. Aliquots of 0.1, 0.5, 1.0, 2.0 and 6.0 ml were removed and added to 0.2% agar for a final volume of 6.0 ml which was added to 24 g liquefied Stoneville diet. The diet had been prepared just prior to the bioassay and was kept in a water bath at about 50 °C until needed. The amended diet was thoroughly mixed and added to 30 wells of a CD International insect diet tray. After the diet had cooled, one neonate *Heliothis virescens* larva was added to each well and the trays were placed in the dark for six days in an environmental chamber (designated Forma LS-133) held at  $24 \pm 2$  °C, 60-65% relative humidity. Sometimes less than 30 wells were infested. Only wells containing one larva were scored.

Because of the high activity of DiPel™, fresh samples and samples incubated without soil were serially diluted first and aliquots were removed for bioassay so that the final concentration was the same as that applied to the soil at the initiation of the experiment.

At the end of the six day exposure period, mortality was assessed. Surviving larvae from each treatment were pooled and weighed.

According to the protocol, with each bioassay run, fresh samples of Cry1F and Dipel with and without soil were to be tested. Due to an oversight, these "fresh" samples were not tested in the first series of bioassays. Subsequent tests showed that the activity of these samples was equal to that of Day 0 samples.

To determine whether the soil by itself contributed to any growth inhibition, soil incubated in the chamber was diluted in the same manner as test samples and bioassayed.

Two types of controls were run. The first consisted of testing the 0.2% solution at the beginning and at the end of each series of bioassays. The second type of control involved filling one well in each 32-well block of the bioassay tray with the 0.2% agar treatment and infesting this control well with one larva at the same time as the other Cry1F- or Dipel- containing wells. This technique was chosen to eliminate variations in control body size related to variation in larvae during the infestation or evaluation phase. Control values agreed very closely with each other, regardless of the control type.

### **Test Organisms**

Names, sources and test stage(s) of test organism: *Heliothis virescens* L., Tobacco Budworm, obtained from French Ag Research, Lamberton, MN. First instar larvae (<24 hours old) were used in the bioassays.

### **Water**

Tap water was supplied by the city of Painesville, Ohio municipal water company. For diet preparation, it was filtered prior to use through a four-stage deionizing Milli-Q Reagent Water System (Millipore Corporation, Bedford, MA 01730) and dispensed according to SOP #17-CO19.

### **Environmental Conditions**

Bioassays were carried out in LS-133 and bioassay trays were kept at  $24 \pm 2^\circ$  C and ca. 75% RH in an environmental chamber in LS-133 (Forma Scientific).

### **Weight Determinations**

Weight determinations were carried out according to SOP #17-C003 using either an AND HA-180M (Serial No. 8801228) balance to weigh samples and larvae or a Mettler BB 240 balance (Serial No. N 37840) to weigh diet ingredients.

### **Statistical Analysis**

For each treatment/exposure length combination, an estimated EC<sub>50</sub> (volume of the solution causing a 50% reduction in growth) was calculated using non-linear regression (PROC NLIN, SAS). The non-linear regression analysis used the mean percent inhibition for each time point for each treatment. Weights from the agar-only treatments were pooled and used as the 0.0 ml

rate. Since there were no consistent growth inhibition effects seen with the soil only samples, the observed larval weights were not corrected for any soil inhibition.

To calculate a DT<sub>50</sub> value for the Cry1F in soil, the natural logarithm of the reciprocal of the EC<sub>50</sub> for Day 0 through Day 14 were regressed using the linear regression feature on a hand-held calculator (Hewlett Packard 15C). The EC<sub>50</sub> value at Day 28 was not used since the change in EC<sub>50</sub> did not appear linear between Day 14 and Day 28. The calculated slope of the regression line was -.221. The equation  $T_{1/2} = 0.693 / (\text{slope})$  was used to calculate the half life.

## RESULTS

Tables 1 and 2 summarize the results for the Cry1F and DiPel™ treatments, respectively. With minor exceptions, the results are based on three or more bioassays for each treatment.

*Table 1: Results of individual bioassays (EC<sub>50</sub> values (ml)) of Cry1F samples incubated in the presence of Drummer Ap loam soil or alone over time.*

EC <sub>50</sub> values (ml) and 95% Confidence Limits at various times								
Treatment	Fresh	0 DAT	3 DAT	5 DAT	7 DAT	10 DAT	14 DAT	28 DAT
Cry1F + Soil	0.090 (0.060-0.121)	0.17 (0.157-0.181)	0.77 (0.439-1.11)	1.04 (0.613-1.48)	1.02 (0.324-1.72)	2.43 (2.09-2.76)	3.26 (2.25-4.28)	3.87 (1.71-6.03)
Cry1F Alone	0.085 (0.067-0.103)	0.039 (0.023-0.054)	0.071 (0.052-0.091)	0.078 (0.065-0.091)	0.059 (0.048-0.069)	0.073 (0.016-0.13)	0.10 (0.083-0.12)	0.097 (0.064-0.13)

EC<sub>50</sub> values for Cry1F powder incubated in soil showed a marked increase over time as shown in Table 1. In the presence of soil, EC<sub>50</sub> values increased from 0.09 ml (fresh Cry1F) to 3.87 ml by Day28, a 43-fold increase. In the absence of soil, the Cry1F EC<sub>50</sub> values ranged from 0.039 ml (Day 0) to 0.10

ml (Day 14). The EC<sub>50</sub> for the "Fresh" Cry1F sample (0.085 ml) was not significantly different from the EC<sub>50</sub> for the Day 28 sample (0.097 ml). The half-life for Cry1F in soil was determined to be 3.13 days.

Due to a technical oversight, a fresh sample of Cry1F tissue powder mixed into soil was not bioassayed with the test samples for the first series of bioassays. Subsequent tests included the fresh Cry1F powder mixed in soil and revealed that there was close agreement of EC<sub>50</sub> values of the fresh sample and Day 0 samples.

DiPel™ was used as an internal standard. The results of bioassays with DiPel™ are presented in Table 2.

**Table 2: Results of individual bioassays (EC<sub>50</sub> values (ml)) of DiPel™ samples incubated in the presence of Drummer Ap loam soil or alone over time.**

EC50 values (ml) and 95% Confidence Limits at various times								
Treatment	Fresh	0 DAT	3 DAT	5 DAT	7 DAT	10 DAT	14 DAT	28 DAT
DiPel™ + Soil	0.34 (0.17-0.51)	0.23 (0.22-0.25)	0.77 (0.54-1.00)	1.45 (1.02-1.87)	0.99 nsr <sup>a</sup>	1.42 nsr <sup>a</sup>	2.58 (1.90-3.26)	2.30 (1.64-2.95)
DiPel™ Alone	0.44 (0.12-0.76)	0.71 (0.58-0.84)	1.18 (0.99-1.36)	2.45 (0.27-4.62)	2.68 (1.84-3.52)	1.98 nsr <sup>a</sup>	1.90 (1.34-2.47)	2.52 (1.68-3.35)

<sup>a</sup> - nsr indicates non-significant regression, where lower 95% confidence limit was < 0.0 ml.

The EC<sub>50</sub> of DiPel™ samples, incubated with soil or alone, decreased slightly over time (Table 2). In both cases, there was a six- to seven-fold increase in the EC<sub>50</sub> by Day 28. The decrease in activity did not appear to be soil dependent since the increase in EC<sub>50</sub>'s was seen for Dipel incubated with or without soil.

## DISCUSSION

This report demonstrated that the Cry1F powder, when mixed with soil, biologically degraded over the 28 day time period of this study. Linear regression provided a  $DT_{50}$  (time needed for a 50% reduction of activity) of 3.13 days. No  $DT_{50}$  value was calculated from the Dipel data since there was no apparent soil mediated degradation.

These results are consistent with previous results (Sims & Holden, Insect Bioassay for Determining Soil Degradation of *Bacillus thuringiensis* subsp. *kurstaki* Cry1A(b) protein in Corn Tissue, *Physiol. Chem. Ecol.* Vol 25(3), pg 659-664). In their study, using essentially the same experimental design, Sims and Holden reported a half-life in soil of 1.6 days. An examination of the data suggest that the half-life of Cry1F in the soil is relatively low (about 3 days). In this study, another Bt microbial product, Dipel™, did not show appreciable decay in the soil environment. Many, if not most, conventional synthetic insecticides have half-lives considerably longer than the 3 days observed in this study.

### **Names of Key Individuals Involved in Study**

<b>Name</b>	<b>Title</b>
W. Ross Halliday	Senior Research Entomologist
Gary J. Misich	Research Biologist

### **Disposition of the Raw Data**

Ricerca, Inc. will maintain, for a minimum of five years, all original study data in Ricerca's archives unless the Sponsor directs otherwise. Upon request, all original study data generated from this study will be shipped to the sponsor, Plant Genetic Systems, for archiving along with the final version of this report via an archive to archive transfer. In this case, the test facility, Ricerca, Inc., will maintain an exact copy of the report and data in its own archives.

**APPENDIX A**

**REDUCED, ORIGINAL COPY OF PROTOCOL AND APPROVAL**

---

Ricerca, Inc.

**PROTOCOL**

**Study Title:**

Environmental Fate of Cry1F Protein  
Incorporated into Soil

**Document Number:** 7569-98-0080-AC-000

**Author:**

W. Ross Halliday, Ph.D., B.C.E.

**Performing Laboratory:**  
Department of Analytical and Biological Services  
Ricerca, Inc.  
7528 Auburn Road  
P. O. Box 1000  
Painesville OH 44077-1000

**Study Sponsor:**  
Mycogen  
5501 Oberlin Drive  
San Diego, CA 92121-1718

---

Page 1 of 16

**Protocol:**

Environmental Fate of Cry1F Protein  
Incorporated into Soil

**Document Number:** 7569-98-0080-AC-000

**DISTRIBUTION**

**Original:**

Ricerca, Inc. Archivist

**Copies:**

Sponsor/Mycogen/ (1) bound, (2) unbound  
QA Files/ (3)  
Analytical and Biological Services Files/(4)  
Study Director/W. R. Halliday(5)

Ricerca, Inc.

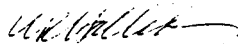
Protocol/Cry1F Soil Decay  
7569-98-0080-AC-000

**PROTOCOL ACCEPTANCE**

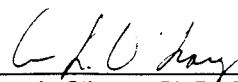
**Study Title:** Environmental Fate of Cry1F Protein  
Incorporated into Soil

**Document Number:** 7569-98-0080-AC-000

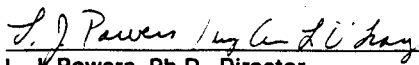
**Performing Laboratory:** Department of Analytical and Biological Services  
Ricerca, Inc.  
7528 Auburn Road  
P. O. Box 1000  
Painesville OH 44077-1000

  
\_\_\_\_\_  
W. Ross Halliday, Ph.D., B.C.E., Study Director  
Senior Research Entomologist  
Analytical and Biological Services

Aug 27 1998  
Date

  
\_\_\_\_\_  
Ann L. O'Leary, Ph.D., Section Head  
Analytical and Biological Services

*monday, Aug 24 11:27 AM*  
Aug 27, 1998  
Date

  
\_\_\_\_\_  
L. J. Powers, Ph.D., Director  
Analytical and Biological Services

August 27, 1998  
Date

**SPONSOR: MYCOGEN**

  
\_\_\_\_\_  
Diane Shanahan/Product Registration Manager  
Mycogen

25 AUGUST 1998  
Date

TABLE OF CONTENTS

	<i>Page</i>
TITLE PAGE .....	1
DISTRIBUTION .....	2
PROTOCOL ACCEPTANCE .....	3
INTRODUCTION .....	5
Study Title .....	5
Sponsor .....	5
Sponsor's Representative .....	5
Test Facility .....	5
Study Director .....	5
Study Number .....	5
Objective .....	6
Justification of Test System .....	6
Schedule of Events .....	6
Retention of Data .....	6
MATERIALS AND METHODS .....	7
Test Substance .....	7
Reference Substance .....	7
EXPERIMENTAL DESIGN .....	7
Overview .....	7
Identification of Test and Control Substances .....	8
Test System .....	8
Addition of Cry 1F to Soil .....	8
Rearing Conditions .....	9
Diet Preparation .....	9
Test Procedure .....	10
Route of Administration and Reason for Choice .....	10
Preparation of the Cry 1F samples .....	10
Mixing the samples into the artificial diet .....	10
Method of Randomization and Bias Control .....	12
PROPOSED STATISTICAL ANALYSIS .....	12
RECORDS TO BE MAINTAINED .....	13
Sample Identification .....	13
Sample Retention and Disposition .....	13
Data Recordkeeping .....	13
Data Presentation .....	14
REPORT .....	14
AMENDMENTS TO THE PROTOCOL .....	15
SAFETY AND HEALTH .....	15
GLP STATEMENT .....	16

## INTRODUCTION

### **STUDY TITLE**

Environmental Fate of Cry1F Protein Incorporated into Soil

### **SPONSOR**

Mycogen  
5501 Oberlin Drive  
San Diego, CA 92121-1718

### **SPONSOR'S REPRESENTATIVE**

Diane Shanahan/Product Registration Manager  
Mycogen  
5501 Oberlin Drive  
San Diego, CA 92121-1718

### **TEST FACILITY**

Ricerca, Inc.  
Analytical and Biological Services  
7528 Auburn Road  
Painesville OH 44077-1000

### **STUDY DIRECTOR**

W. Ross Halliday, Ph.D., B.C.E./Senior Research Entomologist  
Analytical and Biological Services  
Ricerca, Inc.  
7528 Auburn Road  
P.O. Box 1000  
Painesville OH 44077-1000  
Business Phone: (440) 357-3352

### **STUDY NUMBER**

7569-98-0080-AC

**OBJECTIVE**

This study has been designed to determine time dependent bioactivity of Cry1F protein, isolated from a bacterial source, MR872, following incorporation into a typical maize-growing soil under laboratory conditions.

**JUSTIFICATION OF TEST SYSTEM**

*Heliothis virescens* (Tobacco budworm) is highly sensitive to many *Bacillus thuringiensis* based proteins. MacIntosh et al. (J. Ag. Food Chem. 1990, 38:1145-1151) and others have shown that by measuring growth inhibition, sensitivity of insect bioassays with *Bacillus thuringiensis* based products is increased several fold. Sims & Holden (Environmental Entomology 1996, 25:659-664) have used a similar test system to monitor changes in bioactivity with transgenic CryIA(b) protein.

**SCHEDULE OF EVENTS**

Proposed Experimental Starting Date: September 4, 1998

Proposed Completion Date: October 25, 1998

The actual starting and completion dates will be documented in the final report.

**RETENTION OF DATA**

All original raw data and a copy of the report will be retained in the Ricerca, Inc. Corporate Archives, 7528 Auburn Road, Painesville, Ohio.

## MATERIALS AND METHODS

### TEST SUBSTANCE

- Cry1F Protein

Source: Frozen cell paste of *Pseudomonas fluorescens* MR 872 from fermentation lots 970819R, 980722H and 980630C. Production dates: 8/19/98, 7/22/98 and 6/30/98, respectively.

It is the responsibility of the Sponsor to characterize and identify the test substance under GLPs. Original data will be maintained by the Sponsor.

### REFERENCE SUBSTANCE

- DiPel<sup>®</sup> 2X (Lot 15 736PG)

Source: Purchased locally.

## EXPERIMENTAL DESIGN

### OVERVIEW

This study is designed to measure changes in the bioactivity of bacterially expressed Cry1F protein over time following incorporation into soil. Soil and Cry1F will be mixed together, placed in an incubator and sampled over time. The level of Cry1F will be determined by using an insect larval growth inhibition bioassay. For the bioassay, the soil + Cry1F mixture will be serially diluted: aliquots will be added to insect diet in a dose dependent manner to generate dose response lines. Controls will be simultaneously carried out with Cry1F held without soil, and other appropriate conditions as described in further detail below.

**IDENTIFICATION OF TEST AND CONTROL SUBSTANCES**

The test substance for this test will be bacterially expressed Cry1F protein. The Sponsor will provide relevant documentation concerning the origin, identity, strength and storage requirements of the test and control substances for inclusion in the final report.

Commercially available DiPel 2X<sup>®</sup> will be used as a reference standard.

Characterization of the test substance to comply with 40CFR160.105 is the responsibility of the Sponsor. The testing facility requires that the Sponsor identify the chemical and physical properties of material received for testing as thoroughly and completely as possible. The stability of the test and reference substances is not within the scope of this project. A complete description will be included in the final report.

**TEST SYSTEM**

Name	Test Stage	Source
<i>Heliothis virescens</i> Tobacco Budworm	Neonate larvae	USDA-ARS Stoneville, MS

**ADDITION OF CRY1F TO SOIL**

Drummer loam soil (or an equivalent) will be used. The origin and characteristics (% sand, % silt, % clay, % organic matter, pH) will be documented and included in the final report. Characterization will be performed under GLP. Prior to incorporation, the soil will be sieved through a 2 mm sieve to remove extraneous material. Moisture content will be brought to ca. 75% field capacity.

Soil and protein will be mixed together at time zero. The nominal concentration of Cry1F in the soil will be selected based on preliminary experiments. Optimally, the initial rate will provide approximately 70 - 90% growth inhibition and minimal mortality at the most dilute concentrations. Because of experimental variation, the rate chosen may provide some mortality (if the rate is too high) or less than maximal growth inhibition (if the rate is too low).

DiPel 2X<sup>®</sup> will be tested in a manner similar to Cry1F. The application rate and method of application which result in growth inhibition and minimal mortality will be determined based on results from preliminary experiments.

**REARING CONDITIONS**

Eggs will be purchased from commercial sources and shipped by overnight delivery service for next day arrival. Upon arrival at Ricerca, the eggs will be placed in an incubator which is maintained at  $10 \pm 5^\circ\text{C}$  until needed unless they are used immediately. To obtain neonate larvae, eggs will be surface sterilized with a solution consisting of 4% bleach in deionized water containing surfactant, as necessary, and allowed to incubate under conditions needed to provide synchronized hatch.

**DIET PREPARATION**

Insect diet will be prepared. Proposed ingredients, sources and their proportions are listed in the table below.

Name	Source	Catalog No.	Amount Used
Nutri-Soy Flour	Bioserv	1500	$30.12 \pm 0.01$ g
Ground Wheat Germ	Bioserv	1661	$25.68 \pm 0.01$ g
Wesson Salt	ICN Biomedicals	902851	$6.96 \pm 0.01$ g
Sugar	Local Grocery	-	$30.12 \pm 0.01$ g
Methyl Paraben	Bioserv	7685	$720 \pm 5$ mg
Sorbic Acid	Bioserv	6967	$720 \pm 5$ mg
Vitamins	Bioserv	6265	$6.96 \pm 0.01$ g
Agar	Bioserv	7060	$16.40 \pm 0.01$ g
Deionized water	City of Painesville, OH		$682.4 \pm 2$ ml

To dissolve agar for diet preparation, a microwave oven will generally be used. Large batches (>1 liter) of agar will be heated using an autoclave. Agar and deionized water will be mixed in a glass beaker, covered with a lid and placed in the oven. The solution will be heated and stirred occasionally until just below the boiling point. The solution will be allowed to cool and stirred as necessary. After cooling, the agar solution will be added to the rest of the diet ingredients according to manufacturer's instructions. Diet will be used for bioassay generally the day after preparation but can be stored for up to seven days in the refrigerator. Prepared diet will be stored in a refrigerator after it

has cooled. Larger or smaller batches of diet can be prepared using proportional amounts of material.

Deionized water will be used throughout this project.

### **TEST PROCEDURE**

#### **Route of Administration and Reason for Choice**

The activity of the Cry1F protein will be quantitated initially and over time using a larval growth inhibition bioassay with *Heliothis virescens* (Tobacco budworm). The nominal level of Cry1F protein in the soil will be chosen so that maximal growth inhibition and minimal mortality will occur.

Diet bioassays are the bioassay of choice for Cry proteins since these materials act as stomach poisons.

#### **Preparation of the Cry1F samples**

Test materials which are in powdered form will be weighed out. Materials received in other forms will be dispensed using appropriate procedures which will be documented. Samples will be diluted in deionized water or other appropriate solvent and dispensed onto the soil.

#### **Mixing the samples into the artificial diet**

Capped, disposable centrifuge tubes will contain the samples. The cap will contain a small hole to allow gas exchange. Depending on the availability of test and control materials, up to four replicate sets of samples and controls will be prepared. The tubes will be maintained in the dark in a small Plexiglas chamber which maintains high relative humidity. The temperature and humidity within the chamber will be continuously monitored. Six tubes will each contain soil only. These tubes will be weighed at the start and at each sampling time to monitor moisture loss.

A zero time sample will be taken immediately after mixing the soil and Cry1F protein. Subsequent samples will be taken for bioassay after three, five, seven, ten, fourteen and twenty-eight days ( $\pm$  one day) depending on interim results. Should this sampling scheme be altered, such changes will be undertaken in consultation with the Sponsor's representative. Samples will be frozen at  $-70 \pm 5^\circ\text{C}$  if not bioassayed immediately.

For bioassay, the contents of each soil tube will be brought up to a volume of 20 ml with 0.2% agar. Aliquots will be taken and added to liquefied artificial

insect diet. For sample volumes less than 6 ml, 0.2% agar solution will be added to bring the total volume up to 6 ml before it is added to 24 g diet.

Diet will be liquefied using a microwave oven prior to adding the test sample. The bioassay trays, into which the treated diet will be added, will be labeled with the test substance name or code, the concentration, the species being tested, and any other pertinent information. After the diet has cooled, it will be covered to prevent contamination and excessive water loss until the insects are added.

Approximately 30 wells per concentration will be used, each containing one larva. Should more than one larva be added inadvertently, that well will not be scored. After the entire tray has been infested and covered, the tray will be placed in an environmental chamber set at  $25 \pm 3^{\circ}\text{C}$ , and approximately  $80 \pm 20\%$  relative humidity. Six days after the bioassay has been initiated, the surviving larvae will be weighed. All surviving larvae will be weighed together and the average weight per larva will be calculated. Larvae which are not clearly dead or alive will be touched lightly with a probe to determine if they respond. Larvae showing any movement will be classified as alive. No specimens will be kept. Test insects will be autoclaved or frozen and disposed as laboratory waste at the completion of each bioassay.

The units used for reporting the results will be micrograms per gram ( $\mu\text{g/g}$ ) of Cry1F protein in diet or ml of test solution or other appropriate units.

Generally, for bioassays, concentrations should be chosen so that at least two produce  $>50\%$  weight loss, two produce  $<50\%$  weight loss and at least five produce between  $0\%$  and  $100\%$  weight loss. For results to be valid, control mortality should be  $\leq 15\%$ . Due to the experimental nature of the test materials, it may be necessary to override these criteria for this study. If so, this decision will be made by the Study Director in consultation with the Sponsor's Representative.

Duplicate 0.2% agar controls and a soil dilution series (0.1, 0.5, 1.0, 2.0 and 6.0 mls) will be carried out for each bioassay run. In the event that no growth inhibition effect due to the soil alone is seen, the soil dilution series will be discontinued.

Cry1F and Dipel samples, incubated without soil, will be bioassayed as needed to determine whether there is a time dependent loss of activity in the absence of soil.

Enough soil + Cry 1F and soil + Dipel samples will be prepared on Day 0 and immediately frozen at  $-70 \pm 5^{\circ}\text{C}$  so that each bioassay run will have a Day 0 reference time point.

When bioassays are carried out with Cry 1F treated soil samples, fresh samples of Cry 1F + soil and Cry 1F alone will be prepared and bioassayed with the soil exposed samples to provide non-incubated baseline values. Similarly, when Dipel treated soil samples are bioassayed, fresh samples of Dipel + soil and Dipel alone will be prepared and bioassayed with the soil exposed samples to provide non-incubated baseline values.

#### Method of Randomization and Bias Control

All tubes placed into the incubation chamber (Cry 1F + soil, DiPel + soil, soil only, Cry 1F only, DiPel - only and relative humidity tubes) will be arranged in a randomized complete block design within the chamber. The replicate tubes will be bioassayed on different days.

To obtain as random a sample of larvae as possible, sheets containing approximately 500 to 1000 larvae will be placed in a container for hatching. Neonate larvae will be selected without regard to sex for testing based on activity and size. Larvae that are inactive or obviously non-neonate larvae will not be used.

### PROPOSED STATISTICAL ANALYSIS

The total weight of surviving larvae at each dilution will be measured. The average weight per larva will be calculated based on the number of larvae weighed. The threshold for acceptable control mortality is 15%. Should control mortality exceed 15% for a given run, the experiment will be repeated unless the control mortality is considered acceptable by the Sponsor's Representative and Study Director.

Non-linear regression analysis will be used to estimate  $EC_{50}$  and  $EC_{90}$  (concentration (or dilution) of test solution required to cause 50% and 90% reductions in growth) values at each sampling time. Linear regression or other statistical analyses will be performed to estimate the time it takes the sample to decay to  $1/2$  its original strength ( $DT_{50}$ ). The statistical analysis will be documented and included in the final report.

## **RECORDS TO BE MAINTAINED**

### ***SAMPLE IDENTIFICATION***

All samples will be identified by test system, study, nature and date of collection. This information will be located on the sample container or will accompany the sample in a manner that precludes error in the recording and storage of data.

### ***SAMPLE RETENTION AND DISPOSITION***

All test substances will be stored at a temperature compatible with their stability until the final report is issued. The need to reserve samples will be reviewed at that time. If required, the samples will be reserved and their status reviewed periodically. Samples will be disposed only after Quality Assurance and Sponsor agree and in accordance with the appropriate regulations. Samples of the test and reference substances will be reserved at Ricerca, Inc.

The test and control materials will be stored prior to use in an ultra-low temperature freezer maintained at  $-70 \pm 5^{\circ}\text{C}$ , unless otherwise specified by the Sponsor. In accordance with 40 CFR 160.105(d), since the experimental phase of this study is expected to last longer than 4 weeks, a reserve sample of test and reference substances will be taken.

Upon Sponsor's approval of the final report, Ricerca, Inc. will return any unused material and empty containers according to Sponsor's request.

### ***DATA RECORDKEEPING***

All data generated will be correctly labeled and maintained in a project activity file.

Records to be maintained will include, but not be limited, to the following:

- Sample receipt
- Description of experimental detail and methodology
- All relevant raw data
- Correspondence concerning the study

Upon completion of the study report, the entire study file, including all original raw data, will be submitted to the Ricerca Corporate Archives for temporary or permanent storage.

**DATA PRESENTATION**

All operations, data and/or observations will be recorded promptly by signing or initialing and dating by the person conducting the operations, obtaining the data and/or making the observations.

**REPORT**

A final report of the results of this study will be prepared by, or under the supervision of, the Study Director at the conclusion of the study. The report will include, but will not be limited to, the following:

- Name and address of the facility performing the study and the dates on which the study was initiated and was completed, terminated, or discontinued.
- Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
- Statistical methods, if any, employed for analyzing the data.
- The test, control and reference substances identified by name, chemical abstracts service (CAS) number or code number, strength, purity and composition, or other appropriate characteristics.
- If relevant and available, the stability and the solubility of the test, control and reference substances under the conditions of the study.
- A description of the methods used.
- A description of the test system used.
- A description of the experimental design and, if applicable, methods for the control of bias.
- A description of all circumstances that may have affected the quality or integrity of the data.
- The name of the Study Director, the names of other scientists or professionals and the names of all supervisory personnel involved in the study.

- A description of the transformations, calculations or operations performed on the data, a summary and analysis of the data and a statement of the conclusions drawn from the analysis.
- The locations where all specimens, raw data and the final report are to be stored.
- The signed and dated statement by Ricerca, Inc. Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and to Management.
- The signed and dated statement by the Study Director indicating that the study was conducted in compliance with the Good Laboratory Practice Standards 40 CFR Part 160.
- Additions or corrections to the report shall be in the form of an amendment by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and by the Ricerca, Inc. Quality Assurance Unit.

## **AMENDMENTS TO THE PROTOCOL**

All protocol amendments will be expressed in writing, signed and dated by the Study Director and approved by the Study Sponsor.

All agreed upon amendments will be expressed in writing, signed and dated by both the Study Sponsor and the Study Director. Copies of the signed amendments will be returned to the Study Director and appended to the protocol.

## **SAFETY AND HEALTH**

- The Sponsor will provide all relevant health and safety information.
- Laboratory personnel will practice good sanitation and health habits.
- Any health condition of laboratory personnel that may be considered to adversely affect the study will be reported to the Study Director and to the Sponsor.

- Any injury to laboratory personnel during this study, which may be considered related to the test substance, will be reported to the Study Director.
- Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test substance.

### **GLP STATEMENT**

The described study will be conducted in accordance with the "Regulations for Good Laboratory Practice." 40 CFR 160.

**APPENDIX B**

**REDUCED COPY OF CHARACTERIZATION OF TEST  
SUBSTANCE REPORT**

DEC 22 1998 05:16PM


P. 2



**Mycogen**  
3501 Obispo Drive  
San Diego, California 92121  
619 453 8000

**Certificate of Analysis**

Date:	August 30, 1998
Test Substance:	Bacterial Cell Protein, CryIF (truncated)
Lot Number:	1599-39
Analysis Date:	August 28, 1998
Analysist:	Josh Russell
Test Method:	SDS-PAGE with coomassie staining using BSA as standard
Lot Analysis Result:	11.4% cryIF

  
 \_\_\_\_\_  
 Steven L. Evans, Ph.D.  
 Manager, Biochemistry

8/30/98  
 \_\_\_\_\_  
 Date

DEC 22 1998 19:15

PAGE 22

**THIS IS AN EXACT REPRODUCED COPY OF THE ORIGINAL DOCUMENT**

DATE: 12/15/98

SIGNATURE: [Signature]

**APPENDIX C**

**BIOASSAY DATA**

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Total	Number Alive	Percent Control
Soil Fate Assay 1 - Started 10/9/98, Evaluated 10/15/98										
Control 1	0.0	13.1172	13.4333	316.1	11.7	32.3	1	28	27	3.6
Control 2	0.0	13.8453	14.5313	686.0	22.9	-32.3	0	30	30	0.0
Soil Only	0.1	13.3710	13.7267	355.7	12.3	29.0	1	30	29	3.3
Soil Only	0.5	13.4828	14.1626	679.8	22.7	-31.1	0	30	30	0.0
Soil Only	1.0	13.3082	13.7475	439.3	15.1	12.4	1	30	29	3.3
Soil Only	2.0	13.2004	13.6374	437.0	15.1	12.8	1	30	29	3.3
Soil Only	6.0	13.3044	13.6377	333.3	11.1	35.7	0	30	30	0.0
Dipel/No Soil/Day14	0.1	13.3070	13.6613	354.3	12.2	29.3	1	30	29	3.3
Dipel/No Soil/Day14	0.5	13.4009	13.8151	414.2	14.8	14.4	2	30	28	6.7
Dipel/No Soil/Day14	1.0	13.3032	13.5720	268.8	10.8	37.8	4	29	25	13.8
Dipel/No Soil/Day14	2.0	13.4793	13.6001	120.8	5.5	68.2	7	29	22	24.1
Dipel/No Soil/Day14	6.0	13.2123	13.2716	59.3	4.0	77.1	15	30	15	50.0
Dipel/No Soil/Day7	0.1	13.3280	13.8072	479.2	16.0	7.6	0	30	30	0.0
Dipel/No Soil/Day7	0.5	13.3443	13.5486	204.3	8.2	52.7	5	30	25	16.7
Dipel/No Soil/Day7	1.0	13.3574	13.6140	256.6	10.7	38.2	6	30	24	20.0
Dipel/No Soil/Day7	2.0	13.3648	13.5610	196.2	8.2	52.7	6	30	24	20.0
Dipel/No Soil/Day7	6.0	13.2505	13.2939	43.4	2.4	86.1	12	30	18	40.0
Dipel/+ Soil/Day7	0.1	13.4827	13.8310	348.3	11.6	32.8	0	30	30	0.0
Dipel/+ Soil/Day7	0.5	13.3831	13.5559	172.8	6.0	65.5	1	30	29	3.3
Dipel/+ Soil/Day7	1.0	13.2711	13.5736	302.5	10.8	37.5	2	30	28	6.7
Dipel/+ Soil/Day7	2.0	13.3897	13.5741	184.4	7.1	59.0	4	30	26	13.3
Dipel/+ Soil/Day7	6.0	13.3437	13.3927	49.0	2.0	88.7	5	30	25	16.7
Dipel/+ Soil/Day0	0.1	13.4023	13.7147	312.4	10.4	39.8	0	30	30	0.0
Dipel/+ Soil/Day0	0.5	13.3918	13.4985	106.7	3.6	79.4	0	30	30	0.0
Dipel/+ Soil/Day0	1.0	13.4520	13.5457	93.7	3.1	81.9	0	30	30	0.0
Dipel/+ Soil/Day0	2.0	13.4166	13.4645	47.9	1.9	88.9	5	30	25	16.7
Dipel/+ Soil/Day0	6.0	13.8663	13.8881	21.8	0.8	95.1	4	30	26	13.3
Dipel/No Soil/Day0	0.1	13.7365	14.0466	310.1	11.9	31.0	3	29	26	10.3
Dipel/No Soil/Day0	0.5	13.8503	14.0798	229.5	8.2	52.6	2	30	28	6.7
Dipel/No Soil/Day0	1.0	13.8223	13.9827	160.4	5.7	66.9	2	30	28	6.7
Dipel/No Soil/Day0	2.0	13.8481	13.9603	112.2	4.0	76.8	2	30	28	6.7
Dipel/No Soil/Day0	6.0	13.7621	13.7955	33.4	1.8	89.8	11	30	19	36.7
Dipel/+ Soil/Day5	0.1	13.7451	14.1674	422.3	14.1	18.6	0	30	30	0.0
Dipel/+ Soil/Day5	0.5	13.7449	14.1483	403.4	14.4	16.7	2	30	28	6.7
Dipel/+ Soil/Day5	1.0	13.8473	14.1827	335.4	12.4	28.1	3	30	27	10.0
Dipel/+ Soil/Day5	2.0	13.8138	13.9670	153.2	5.3	69.4	1	30	29	3.3
Dipel/+ Soil/Day5	6.0	13.6491	13.7183	69.2	2.9	83.3	6	30	24	20.0
Dipel/No Soil/Day5	0.1	13.8230	14.3415	518.5	19.2	-11.1	2	29	27	6.9
Dipel/No Soil/Day5	0.5	13.9610	14.3980	437.0	17.5	-1.1	5	30	25	16.7
Dipel/No Soil/Day5	1.0	13.8187	14.0942	275.5	11.5	33.6	6	30	24	20.0
Dipel/No Soil/Day5	2.0	13.7542	14.0271	272.9	12.4	28.2	8	30	22	26.7
Dipel/No Soil/Day5	6.0	13.8429	13.8836	40.7	2.9	83.2	16	30	14	53.3
Dipel/+ Soil/Day14	0.1	13.6481	14.2339	585.8	20.2	-16.9	1	30	29	3.3
Dipel/+ Soil/Day14	0.5	13.8112	14.3931	581.9	20.8	-20.2	2	30	28	6.7
Dipel/+ Soil/Day14	1.0	13.7836	14.2100	426.4	14.7	14.9	1	30	29	3.3
Dipel/+ Soil/Day14	2.0	13.9606	14.3274	366.8	14.1	18.4	4	30	26	13.3
Dipel/+ Soil/Day14	6.0	13.8704	14.0516	181.2	7.0	59.7	4	30	26	13.3
Soil Fate Assay 2 - Started 10/16/98, Evaluated 10/22/98										
Control 1	0.0	13.3711	14.0691	698.0	23.3	7.2	0	30	30	0.0
Control 2	0.0	13.4567	14.2629	806.2	26.9	-7.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	0.1	13.3119	13.6289	317.0	10.9	56.4	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day0	0.5	13.3317	13.4830	151.3	5.2	79.2	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day0	1.0	13.3267	13.4269	100.2	3.3	86.7	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	2.0	13.2339	13.2733	39.4	1.4	94.6	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day0	6.0	13.3610	13.3631	2.1	0.1	99.6	10	30	20	33.3
Bacterial Cry1F/No Soil/Day0	0.1	13.3792	13.6159	236.7	7.9	68.5	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	0.5	13.3434	13.3970	53.6	1.8	92.9	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	1.0	13.2328	13.2595	26.7	1.1	95.7	4	29	25	13.8
Bacterial Cry1F/No Soil/Day0	2.0	13.2245	13.2253	0.8	0.0	99.8	11	30	19	36.7
Bacterial Cry1F/No Soil/Day0	6.0	13.3311	13.3342	3.1	0.6	97.5	25	30	5	83.3
Bactenal Cry1F/+ Soil/Day7	0.1	13.4471	14.2389	791.8	26.4	-5.3	0	30	30	0.0

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Total	Number Alive	Percent Control
Bacterial Cry1F/+ Soil/Day7	0.5	13.3693	13.8778	508.5	17.0	32.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	1.0	13.2283	13.6688	440.5	14.7	41.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	2.0	13.4280	13.6449	216.9	7.2	71.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	6.0	13.4200	13.5932	173.2	5.8	77.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	0.1	13.3413	13.9760	634.7	21.2	15.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	0.5	13.3388	13.9613	622.5	20.8	17.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	1.0	13.3331	13.8406	507.5	16.9	32.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	2.0	13.3343	13.6790	344.7	11.9	52.6	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day10	6.0	13.3823	13.5487	166.4	5.5	77.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	0.1	13.3825	14.1583	775.8	26.8	-6.7	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day28	0.5	13.4096	14.2405	830.9	28.7	-14.3	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day28	1.0	13.3758	14.0616	685.8	22.9	8.8	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	2.0	13.2963	13.8753	579.0	19.3	23.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	6.0	13.2518	13.6712	419.4	14.5	42.3	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day14	0.1	13.3548	14.1298	775.0	26.7	-6.6	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day14	0.5	13.2702	14.0848	814.6	27.2	-8.3	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	1.0	13.3707	13.9839	613.2	22.7	9.4	2	29	27	6.9
Bacterial Cry1F/+ Soil/Day14	2.0	13.3428	13.7991	456.3	16.9	32.6	1	28	27	3.6
Bacterial Cry1F/+ Soil/Day14	6.0	13.3789	13.6848	305.9	10.9	56.4	0	28	28	0.0
Bacterial Cry1F/+ Soil/Day3	0.1	13.3858	14.1260	740.2	24.7	1.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.5	13.4110	13.8346	423.6	14.6	41.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day3	1.0	13.4437	13.8455	401.8	13.9	44.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day3	2.0	13.3387	13.4715	132.8	4.6	81.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day3	6.0	13.3417	13.4592	117.5	4.1	83.8	1	30	29	3.3
Soil Only/Day3	0.1	13.2728	14.2268	954.0	31.8	-26.8	0	30	30	0.0
Soil Only/Day3	0.5	13.3112	14.1613	850.1	29.3	-16.9	1	30	29	3.3
Soil Only/Day3	1.0	13.3948	14.3117	916.9	31.6	-26.1	1	30	29	3.3
Soil Only/Day3	2.0	13.4044	13.7778	377.4	13.0	48.1	0	29	29	0.0
Soil Only/Day3	6.0	13.2240	14.0401	816.1	28.1	-12.3	0	29	29	0.0
Bacterial Cry1F/No Soil/Day10	0.1	13.3845	13.5077	123.2	8.2	67.2	1	16	15	6.3
Bacterial Cry1F/No Soil/Day10	0.5	13.3330	13.3749	41.9	2.8	88.9	1	16	15	6.3
Bacterial Cry1F/No Soil/Day10	1.0	13.4301	13.4335	3.4	0.3	98.8	5	16	11	31.3
Bacterial Cry1F/No Soil/Day10	2.0	13.3622	13.3646	2.4	0.2	99.0	6	16	10	37.5
Bacterial Cry1F/No Soil/Day10	6.0	13.2238	13.2315	7.7	7.7	69.3	15	16	1	93.8
Bacterial Cry1F/No Soil/Day7	0.1	13.2762	13.4988	222.6	7.4	70.4	0	30	30	0.0
Bacterial Cry1F/No Soil/Day7	0.5	13.3510	13.4922	141.2	4.7	81.2	0	30	30	0.0
Bacterial Cry1F/No Soil/Day7	1.0	13.3392	13.3972	58.0	2.5	89.9	7	30	23	23.3
Bacterial Cry1F/No Soil/Day7	2.0	13.2289	13.2458	16.9	0.9	96.5	10	29	19	34.5
Bacterial Cry1F/No Soil/Day7	6.0	13.2905	13.2949	4.4	0.4	98.4	19	30	11	63.3
Bacterial Cry1F/No Soil/Day5	0.1	13.3537	13.7309	377.2	12.6	49.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day5	0.5	13.2332	13.3623	129.1	4.5	82.2	1	30	29	3.3
Bacterial Cry1F/No Soil/Day5	1.0	13.3338	13.3673	33.5	1.3	94.9	4	30	26	13.3
Bacterial Cry1F/No Soil/Day5	2.0	13.3362	13.3425	6.3	0.4	98.4	14	30	16	46.7
Bacterial Cry1F/No Soil/Day5	6.0	13.4331	13.4312	-1.9	-0.2	100.8	20	30	10	66.7
Bacterial Cry1F/+ Soil/Day5	0.1	13.3791	14.3657	986.6	32.9	-31.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	0.5	13.3894	13.7043	314.9	10.5	58.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	1.0	13.3254	13.7604	435.0	14.5	42.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	2.0	13.3694	13.5294	160.0	5.5	78.0	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day5	6.0	13.2669	13.3896	122.7	4.5	81.9	1	28	27	3.6
Bacterial Cry1F/No Soil/Day14	0.1	13.3522	13.7074	355.2	11.8	52.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day14	0.5	13.2156	13.3133	97.7	3.9	84.4	2	27	25	7.4
Bacterial Cry1F/No Soil/Day14	1.0	13.4212	13.4769	55.7	2.0	92.1	2	30	28	6.7
Bacterial Cry1F/No Soil/Day14	2.0	13.3120	13.3204	8.4	0.5	98.1	12	30	18	40.0
Bacterial Cry1F/No Soil/Day14	6.0	13.3269	13.3247	-2.2	-0.3	101.1	22	30	8	73.3
Bacterial Cry1F/No Soil/Day3	0.1	13.4296	13.7050	275.4	9.2	63.4	0	30	30	0.0
Bacterial Cry1F/No Soil/Day3	0.5	13.2184	13.2864	68.0	2.5	90.0	2	29	27	6.9
Bacterial Cry1F/No Soil/Day3	1.0	13.3726	13.4093	36.7	1.7	93.3	7	29	22	24.1
Bacterial Cry1F/No Soil/Day3	2.0	13.3335	13.3404	6.9	0.5	98.2	15	30	15	50.0
Bacterial Cry1F/No Soil/Day3	6.0	13.2218	13.2248	3.0	0.2	99.1	16	30	14	53.3
Soil Fate Assay 3 - Started 10/21/98, Evaluated 10/27/98										
Control 1	0.0	13.6830	14.5053	822.3	24.9	3.4	0	33	33	0.0
Control 2	0.0	13.7610	14.6088	847.8	28.3	-9.6	0	30	30	0.0

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Total	Number Alive	Percent Control
Control 3	0.0	13.2333	14.9271	1693.8	24.2	6.2	0	70	70	0.0
Dipel/No Soil/Day10	0.1	13.7522	14.5535	801.3	28.6	-11.0	2	30	28	6.7
Dipel/No Soil/Day10	0.5	13.7984	14.4999	701.5	24.2	6.2	1	30	29	3.3
Dipel/No Soil/Day10	1.0	13.7158	14.2225	506.7	22.0	14.6	7	30	23	23.3
Dipel/No Soil/Day10	2.0	13.7856	14.2009	415.3	19.8	23.3	9	30	21	30.0
Dipel/No Soil/Day10	6.0	13.8338	13.9159	82.1	6.3	75.5	17	30	13	56.7
Dipel/+ Soil/Day10	0.1	13.7608	14.4895	728.7	24.3	5.8	0	30	30	0.0
Dipel/+ Soil/Day10	0.5	13.7575	14.3755	618.0	20.6	20.1	0	30	30	0.0
Dipel/+ Soil/Day10	1.0	14.1536	14.5879	434.3	15.5	39.9	2	30	28	6.7
Dipel/+ Soil/Day10	2.0	13.7116	13.9483	236.7	9.5	63.3	5	30	25	16.7
Dipel/+ Soil/Day10	6.0	13.7117	13.8026	90.9	5.0	80.4	8	26	18	30.8
Dipel/+ Soil/Day7	0.1	13.7808	14.4699	689.1	23.0	10.9	0	30	30	0.0
Dipel/+ Soil/Day7	0.5	13.8211	14.2445	423.4	15.7	39.2	3	30	27	10.0
Dipel/+ Soil/Day7	1.0	13.7429	14.2389	496.0	17.1	33.7	1	30	29	3.3
Dipel/+ Soil/Day7	2.0	13.7974	14.0275	230.1	8.2	68.1	2	30	28	6.7
Dipel/+ Soil/Day7	6.0	13.7685	13.8428	74.3	3.1	88.0	6	30	24	20.0
Dipel/+ Soil/Day3	0.1	13.9080	14.5598	651.8	21.7	15.8	0	30	30	0.0
Dipel/+ Soil/Day3	0.5	13.7232	14.0795	356.3	12.3	52.4	1	30	29	3.3
Dipel/+ Soil/Day3	1.0	13.7632	14.1174	354.2	13.1	49.1	3	30	27	10.0
Dipel/+ Soil/Day3	2.0	13.7581	13.8694	111.3	4.8	81.2	7	30	23	23.3
Dipel/+ Soil/Day3	6.0	13.7429	13.8057	62.8	2.4	90.6	4	30	26	13.3
Dipel/+ Soil/Day14	0.1	13.8753	14.4446	569.3	20.3	21.2	2	30	28	6.7
Dipel/+ Soil/Day14	0.5	13.7337	14.2488	515.1	17.2	33.4	0	30	30	0.0
Dipel/+ Soil/Day14	1.0	13.7629	14.3252	562.3	18.7	27.3	0	30	30	0.0
Dipel/+ Soil/Day14	2.0	13.7550	14.0025	247.5	9.5	63.1	4	30	26	13.3
Dipel/+ Soil/Day14	6.0	13.7524	13.8436	91.2	3.8	85.3	5	29	24	17.2
Dipel/No Soil/Day28	0.1	13.7248	14.3398	615.0	21.2	17.8	0	29	29	0.0
Dipel/No Soil/Day28	0.5	13.8150	14.3474	532.4	18.4	28.8	1	30	29	3.3
Dipel/No Soil/Day28	1.0	13.6889	14.0902	401.3	14.9	42.4	3	30	27	10.0
Dipel/No Soil/Day28	2.0	13.7683	14.0128	244.5	12.9	50.1	11	30	19	36.7
Dipel/No Soil/Day28	6.0	13.6793	13.7714	92.1	5.4	79.0	13	30	17	43.3
Dipel/No Soil/Day5	0.1	13.6892	14.3145	625.3	20.8	19.2	0	30	30	0.0
Dipel/No Soil/Day5	0.5	14.1193	14.6985	579.2	20.0	22.6	1	30	29	3.3
Dipel/No Soil/Day5	1.0	13.8840	14.3768	492.8	17.6	31.8	2	30	28	6.7
Dipel/No Soil/Day5	2.0	13.8432	14.2306	387.4	14.3	44.4	3	30	27	10.0
Dipel/No Soil/Day5	6.0	13.8223	13.9190	96.7	6.4	75.0	15	30	15	50.0
Dipel/No Soil/Day14	0.1	13.7387	14.5091	770.4	25.7	0.4	0	30	30	0.0
Dipel/No Soil/Day14	0.5	13.7099	14.2791	569.2	19.6	23.9	1	30	29	3.3
Dipel/No Soil/Day14	1.0	13.7893	14.2892	499.9	19.2	25.5	4	30	26	13.3
Dipel/No Soil/Day14	2.0	13.9373	14.2507	313.4	12.5	51.4	5	30	25	16.7
Dipel/No Soil/Day14	6.0	13.7515	13.9423	190.8	10.0	61.1	11	30	19	36.7
Dipel/+ Soil/Day0	0.1	13.6999	14.2802	580.3	19.3	25.0	0	30	30	0.0
Dipel/+ Soil/Day0	0.5	13.6858	13.9969	311.1	10.4	59.8	0	30	30	0.0
Dipel/+ Soil/Day0	1.0	13.7727	13.9471	174.4	5.8	77.5	0	30	30	0.0
Dipel/+ Soil/Day0	2.0	13.7358	13.8159	80.1	3.0	88.5	3	30	27	10.0
Dipel/+ Soil/Day0	6.0	13.7998	13.8293	29.5	1.1	95.8	3	30	27	10.0
Dipel/+ Soil/Day28	0.1	13.7493	14.5622	812.9	27.1	-5.1	0	30	30	0.0
Dipel/+ Soil/Day28	0.5	13.8148	14.5567	741.9	25.6	0.8	1	30	29	3.3
Dipel/+ Soil/Day28	1.0	13.7303	14.2422	511.9	19.0	26.5	3	30	27	10.0
Dipel/+ Soil/Day28	2.0	13.8235	14.2473	423.8	16.3	36.8	4	30	26	13.3
Dipel/+ Soil/Day28	6.0	13.7592	13.8453	86.1	3.2	87.6	3	30	27	10.0
Dipel/No Soil/Day3	0.1	13.8112	14.4684	657.2	23.5	9.0	2	30	28	6.7
Dipel/No Soil/Day3	0.5	13.7219	14.3065	584.6	19.5	24.4	0	30	30	0.0
Dipel/No Soil/Day3	1.0	13.7585	14.0914	332.9	12.8	50.4	4	30	26	13.3
Dipel/No Soil/Day3	2.0	13.6944	13.8501	155.7	6.2	75.9	5	30	25	16.7
Dipel/No Soil/Day3	6.0	13.7290	13.7962	67.2	2.6	90.0	4	30	26	13.3
Dipel/No Soil/Day0	0.1	13.6918	14.4043	712.5	24.6	4.7	1	30	29	3.3
Dipel/No Soil/Day0	0.5	13.8913	14.3993	508.0	18.8	27.1	3	30	27	10.0
Dipel/No Soil/Day0	1.0	13.9396	14.2938	354.2	12.2	52.6	1	30	29	3.3
Dipel/No Soil/Day0	2.0	13.8437	13.9725	128.6	5.4	79.2	6	30	24	20.0
Dipel/No Soil/Day0	6.0	13.6880	13.7386	50.6	1.9	92.5	4	30	26	13.3
Dipel/+ Soil/Day5	0.1	13.7196	14.5469	827.3	27.6	-6.9	0	30	30	0.0

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Dipel/+ Soil/Day5	0.5	13.8692	14.4628	593.6	22.0	14.8	3	30	27	10.0
Dipel/+ Soil/Day5	1.0	13.7087	14.1203	411.6	17.9	30.6	7	30	23	23.3
Dipel/+ Soil/Day5	2.0	13.7529	13.9278	174.9	6.7	73.9	4	30	26	13.3
Dipel/+ Soil/Day5	6.0	13.9332	14.0254	92.2	3.5	86.3	4	30	26	13.3
Soil Only/Day5	0.1	13.6922	14.8288	1136.6	37.9	-46.9	0	30	30	0.0
Soil Only/Day5	0.5	13.8470	14.8571	1010.1	33.7	-30.5	0	30	30	0.0
Soil Only/Day5	1.0	13.7923	14.8709	1078.6	36.0	-39.4	0	30	30	0.0
Soil Only/Day5	2.0	13.7856	14.6233	837.7	27.9	-8.3	0	30	30	0.0
Soil Only/Day5	6.0	13.7982	14.3484	550.2	19.0	26.4	0	29	29	0.0
Dipel/No Soil/Day7	0.1	13.7588	14.3819	623.1	21.5	16.7	1	30	29	3.3
Dipel/No Soil/Day7	0.5	13.7636	14.3559	592.3	22.8	11.7	4	30	26	13.3
Dipel/No Soil/Day7	1.0	13.7455	14.2766	531.1	21.2	17.6	5	30	25	16.7
Dipel/No Soil/Day7	2.0	13.7993	14.2266	427.3	15.8	38.6	3	30	27	10.0
Dipel/No Soil/Day7	6.0	13.8253	13.9359	110.6	6.9	73.2	14	30	16	46.7
Soil Fate Assay 4 - Started 10/28/98, Evaluated 11/3/98										
Control 1	0.0	13.7641	14.5104	746.3	25.7	8.5	2	31	29	6.5
Control 2	0.0	14.1118	15.0854	973.6	32.5	-15.4	0	30	30	0.0
Control 3	0.0	13.7580	15.6937	1935.7	26.2	7.0	1	75	74	1.3
Bacterial Cry1F/No Soil/Day3	0.1	13.6778	13.9299	252.1	8.4	70.1	0	30	30	0.0
Bacterial Cry1F/No Soil/Day3	0.5	13.7419	13.8031	61.2	2.2	92.2	2	30	28	6.7
Bacterial Cry1F/No Soil/Day3	1.0	14.1555	14.1763	20.8	1.0	96.5	9	30	21	30.0
Bacterial Cry1F/No Soil/Day3	2.0	13.8972	13.9074	10.2	0.6	97.9	13	30	17	43.3
Bacterial Cry1F/No Soil/Day3	6.0	14.0953	14.0989	3.6	1.8	93.6	28	30	2	93.3
Bacterial Cry1F/+ Soil/Day5	0.1	13.7568	14.4749	718.1	23.9	14.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	0.5	13.6919	14.3669	675.0	22.5	20.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	1.0	13.8104	14.1263	315.9	10.5	62.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	2.0	13.7471	14.0070	259.9	8.7	69.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	6.0	13.8231	13.8894	66.3	2.2	92.1	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	0.1	13.7316	14.0815	349.9	11.7	58.5	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	0.5	13.7486	13.8259	77.3	2.6	90.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	1.0	13.8881	13.9117	23.6	1.0	96.5	6	30	24	20.0
Bacterial Cry1F/No Soil/Day28	2.0	13.8504	13.8584	8.0	0.7	97.4	19	30	11	63.3
Bacterial Cry1F/No Soil/Day28	6.0	13.7287	13.7310	2.3	2.3	91.8	28	29	1	96.6
Bacterial Cry1F/No Soil/Day0	0.1	13.6925	13.9215	229.0	7.6	72.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	0.5	13.7272	13.7630	35.8	1.3	95.5	2	30	28	6.7
Bacterial Cry1F/No Soil/Day0	1.0	13.8239	13.8612	37.3	1.8	93.7	9	30	21	30.0
Bacterial Cry1F/No Soil/Day0	2.0	13.8602	13.8666	6.4	0.5	98.2	17	30	13	56.7
Bacterial Cry1F/No Soil/Day0	6.0	13.7438	13.7445	0.7	0.1	99.7	22	30	8	73.3
Bacterial Cry1F/+ Soil/Day10	0.1	13.8387	14.6646	825.9	27.5	2.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	0.5	13.7356	14.3628	627.2	21.6	23.1	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day10	1.0	13.7451	14.3670	621.9	20.7	26.3	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	2.0	13.8884	14.1769	288.5	9.6	65.8	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	6.0	13.7584	13.9520	193.6	6.5	77.0	0	30	30	0.0
Bacterial Cry1F/No Soil/Day14	0.1	13.8079	14.0957	287.8	9.9	64.7	1	30	29	3.3
Bacterial Cry1F/No Soil/Day14	0.5	13.7413	13.8242	82.9	3.0	89.5	2	30	28	6.7
Bacterial Cry1F/No Soil/Day14	1.0	13.6919	13.7211	29.2	1.5	94.5	11	30	19	36.7
Bacterial Cry1F/No Soil/Day14	2.0	13.7670	13.7711	4.1	0.3	98.9	17	30	13	56.7
Bacterial Cry1F/No Soil/Day14	6.0	13.7224	13.7260	3.6	0.6	97.9	24	30	6	80.0
Bacterial Cry1F/+ Soil/Day7	0.1	13.6956	14.5404	844.8	28.2	-0.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	0.5	13.7379	14.4803	742.4	24.7	12.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	1.0	13.7245	14.2193	494.8	17.1	39.3	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day7	2.0	13.8303	14.1127	282.4	9.4	66.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	6.0	13.7677	13.9027	135.0	4.5	84.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.1	14.1266	14.9227	796.1	26.5	5.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.5	13.7427	14.1319	389.2	13.0	53.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	1.0	13.7739	14.1776	403.7	13.5	52.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	2.0	13.7279	13.8708	142.9	4.9	82.5	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day3	6.0	13.8324	13.8772	44.8	1.7	93.9	4	30	26	13.3
Bacterial Cry1F/+ Soil/Day14	0.1	13.8185	14.5484	729.9	25.2	10.5	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day14	0.5	13.7363	14.4397	703.4	23.4	16.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	1.0	13.7960	14.3963	600.3	20.7	26.4	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day14	2.0	13.8743	14.2753	401.0	13.8	50.8	1	30	29	3.3

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Bacterial Cry1F/+ Soil/Day14	6.0	13.7517	13.9719	220.2	7.9	72.0	2	30	28	6.7
Bacterial Cry1F/No Soil/Day10	0.1	13.7436	14.0355	291.9	9.7	65.4	0	30	30	0.0
Bacterial Cry1F/No Soil/Day10	0.5	13.7891	13.8583	69.2	2.9	89.7	4	28	24	14.3
Bacterial Cry1F/No Soil/Day10	1.0	13.7296	13.7658	36.2	1.8	93.6	9	29	20	31.0
Bacterial Cry1F/No Soil/Day10	2.0	14.1477	14.1660	18.3	1.1	95.9	14	30	16	46.7
Bacterial Cry1F/No Soil/Day10	6.0	13.7873	13.7875	0.2	0.0	99.9	24	29	5	82.8
Bacterial Cry1F/No Soil/Day5	0.1	13.7086	14.0010	292.4	10.4	62.9	2	30	28	6.7
Bacterial Cry1F/No Soil/Day5	0.5	13.7885	13.8620	73.5	2.7	90.3	3	30	27	10.0
Bacterial Cry1F/No Soil/Day5	1.0	13.7250	13.7669	41.9	2.6	90.7	13	29	16	44.8
Bacterial Cry1F/No Soil/Day5	2.0	13.8487	13.8594	10.7	0.7	97.6	14	30	16	46.7
Bacterial Cry1F/No Soil/Day5	6.0	14.1235	14.1332	9.7	2.4	91.4	26	30	4	86.7
Soil Only/Day7	0.1	13.9376	14.7027	765.1	25.5	9.3	0	30	30	0.0
Soil Only/Day7	0.5	13.7292	14.2725	543.3	20.1	28.4	3	30	27	10.0
Soil Only/Day7	1.0	13.7366	14.5786	842.0	28.1	0.2	0	30	30	0.0
Soil Only/Day7	2.0	13.7693	14.5575	788.2	27.2	3.3	1	30	29	3.3
Soil Only/Day7	6.0	13.8124	14.6535	841.1	30.0	-6.8	1	29	28	3.4
Bacterial Cry1F/No Soil/Day7	0.1	13.7044	14.0328	328.4	11.7	58.3	2	30	28	6.7
Bacterial Cry1F/No Soil/Day7	0.5	13.6657	13.7426	76.9	3.5	87.6	8	30	22	26.7
Bacterial Cry1F/No Soil/Day7	1.0	13.7784	13.8022	23.8	1.6	94.4	15	30	15	50.0
Bacterial Cry1F/No Soil/Day7	2.0	14.1212	14.1332	12.0	1.2	95.7	19	29	10	65.5
Bacterial Cry1F/No Soil/Day7	6.0	13.7579	13.7605	2.6	0.7	97.7	26	30	4	86.7
Bacterial Cry1F/+ Soil/Day28	0.1	13.9340	14.8882	954.2	31.8	-13.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	0.5	13.7250	14.7392	1014.2	33.8	-20.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	1.0	13.7458	14.2800	534.2	21.4	24.0	3	28	25	10.7
Bacterial Cry1F/+ Soil/Day28	2.0	13.7706	14.2705	499.9	17.9	36.5	1	29	28	3.4
Bacterial Cry1F/+ Soil/Day28	6.0	13.8702	14.0111	140.9	4.9	82.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day0	0.1	13.6609	14.2065	545.6	19.5	30.7	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day0	0.5	13.8013	13.9506	149.3	5.3	81.0	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day0	1.0	13.8257	13.8668	41.1	1.9	93.4	8	30	22	26.7
Bacterial Cry1F/+ Soil/Day0	2.0	13.7633	13.7820	18.7	1.9	93.3	20	30	10	66.7
Bacterial Cry1F/+ Soil/Day0	6.0	13.8517	13.8599	8.2	0.7	97.6	16	28	12	57.1
Soil Fate Assay 5 - Started 10/30/98. Evaluated 11/5/98										
Control 1	0.0	13.6903	14.5242	833.9	29.8	-25.2	1	29	28	3.4
Control 2	0.0	13.7266	14.2414	514.8	18.4	22.7	2	30	28	6.7
Control 3	0.0	13.7004	15.4846	1784.2	23.2	2.6	2	79	77	2.5
Dipel/+ Soil/Day14	0.1	13.8238	14.6790	855.2	28.5	-19.9	0	30	30	0.0
Dipel/+ Soil/Day14	0.5	13.7856	14.4601	674.5	24.1	-1.3	2	30	28	6.7
Dipel/+ Soil/Day14	1.0	13.7884	14.3918	603.4	22.3	6.0	3	30	27	10.0
Dipel/+ Soil/Day14	2.0	13.9240	14.3215	397.5	13.3	44.3	0	30	30	0.0
Dipel/+ Soil/Day14	6.0	13.7442	13.9009	156.7	5.4	77.3	0	29	29	0.0
Dipel/No Soil/Day3	0.1	13.7911	14.6048	813.7	29.1	-22.2	2	30	28	6.7
Dipel/No Soil/Day3	0.5	14.1159	14.8018	685.9	22.9	3.9	0	30	30	0.0
Dipel/No Soil/Day3	1.0	14.1567	14.6012	444.5	17.1	28.1	4	30	26	13.3
Dipel/No Soil/Day3	2.0	13.7149	13.9466	231.7	10.1	57.6	7	30	23	23.3
Dipel/No Soil/Day3	6.0	13.7748	13.8421	67.3	2.9	87.7	7	30	23	23.3
Dipel/No Soil/Day28	0.1	13.9194	14.8555	936.1	32.3	-35.7	1	30	29	3.3
Dipel/No Soil/Day28	0.5	13.6828	14.3533	670.5	23.9	-0.7	2	30	28	6.7
Dipel/No Soil/Day28	1.0	13.8082	14.4206	612.4	24.5	-3.0	4	29	25	13.8
Dipel/No Soil/Day28	2.0	13.8663	14.2309	364.6	17.4	27.0	9	30	21	30.0
Dipel/No Soil/Day28	6.0	13.7590	13.9112	152.2	9.0	62.4	13	30	17	43.3
Soil Only/Day10	0.1	13.7126	14.5816	869.0	29.0	-21.8	0	30	30	0.0
Soil Only/Day10	0.5	13.9023	14.7057	803.4	26.8	-12.6	0	30	30	0.0
Soil Only/Day10	1.0	13.8161	14.7696	953.5	31.8	-33.7	0	30	30	0.0
Soil Only/Day10	2.0	13.7518	14.4879	736.1	26.3	-10.6	1	29	28	3.4
Soil Only/Day10	6.0	13.7768	14.5270	750.2	25.9	-8.8	0	29	29	0.0
Dipel/No Soil/Day7	0.1	13.7139	14.5473	833.4	27.8	-16.8	0	30	30	0.0
Dipel/No Soil/Day7	0.5	14.1388	14.9112	772.4	27.6	-16.0	2	30	28	6.7
Dipel/No Soil/Day7	1.0	14.1623	14.7365	574.2	23.9	-0.6	6	30	24	20.0
Dipel/No Soil/Day7	2.0	14.1599	14.5402	380.3	16.5	30.5	7	30	23	23.3
Dipel/No Soil/Day7	6.0	13.9185	14.0278	109.3	6.8	71.3	14	30	16	46.7
Dipel/+ Soil/Day28	0.1	13.6683	14.5123	844.0	29.1	-22.4	1	30	29	3.3
Dipel/+ Soil/Day28	0.5	13.7816	14.5553	773.7	26.7	-12.2	1	30	29	3.3

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Dipel/+ Soil/Day28	1.0	13.7183	14.3429	624.6	22.3	6.2	2	30	28	6.7
Dipel/+ Soil/Day28	2.0	13.7816	14.1067	325.1	12.0	49.4	3	30	27	10.0
Dipel/+ Soil/Day28	6.0	13.8596	14.0314	171.8	6.9	71.1	4	29	25	13.8
Dipel/+ Soil/Day3	0.1	13.7674	14.5099	742.5	28.6	-20.1	2	28	26	7.1
Dipel/+ Soil/Day3	0.5	13.7772	14.2092	432.0	16.6	30.1	4	30	26	13.3
Dipel/+ Soil/Day3	1.0	13.8491	14.2113	362.2	12.5	47.5	1	30	29	3.3
Dipel/+ Soil/Day3	2.0	13.6984	13.8707	172.3	6.2	74.1	2	30	28	6.7
Dipel/+ Soil/Day3	6.0	13.7069	13.7619	55.0	2.2	90.7	5	30	25	16.7
Dipel/+ Soil/Day5	0.1	13.7468	14.5469	800.1	26.7	-12.2	0	30	30	0.0
Dipel/+ Soil/Day5	0.5	14.1628	14.6890	526.2	18.8	21.0	2	30	28	6.7
Dipel/+ Soil/Day5	1.0	13.6978	14.1106	412.8	15.3	35.7	3	30	27	10.0
Dipel/+ Soil/Day5	2.0	13.8916	14.1738	282.2	10.5	56.0	3	30	27	10.0
Dipel/+ Soil/Day5	6.0	13.8109	13.8702	59.3	2.6	89.2	7	30	23	23.3
Dipel/No Soil/Day0	0.1	13.6916	14.4255	733.9	24.5	-2.9	0	30	30	0.0
Dipel/No Soil/Day0	0.5	13.6617	14.0744	412.7	14.7	38.0	1	29	28	3.4
Dipel/No Soil/Day0	1.0	13.7301	13.9944	264.3	9.4	60.3	2	30	28	6.7
Dipel/No Soil/Day0	2.0	13.7201	13.8416	121.5	4.7	80.3	3	29	26	10.3
Dipel/No Soil/Day0	6.0	13.7164	13.7863	69.9	2.8	88.2	5	30	25	16.7
Dipel/+ Soil/Day7	0.1	13.7234	14.5590	835.6	27.9	-17.1	0	30	30	0.0
Dipel/+ Soil/Day7	0.5	13.7451	14.1796	434.5	15.5	34.7	2	30	28	6.7
Dipel/+ Soil/Day7	1.0	13.7707	14.0021	231.4	10.5	55.8	8	30	22	26.7
Dipel/+ Soil/Day7	2.0	13.7212	14.0141	292.9	11.3	52.6	4	30	26	13.3
Dipel/+ Soil/Day7	6.0	13.9279	14.0399	112.0	4.7	80.4	6	30	24	20.0
Dipel/+ Soil/Day0	0.1	14.1394	14.6113	471.9	17.5	26.5	3	30	27	10.0
Dipel/+ Soil/Day0	0.5	13.7218	13.9506	228.8	8.2	65.6	1	29	28	3.4
Dipel/+ Soil/Day0	1.0	13.7737	13.8907	117.0	4.5	81.1	3	29	26	10.3
Dipel/+ Soil/Day0	2.0	13.7231	13.7712	48.1	1.9	91.9	5	30	25	16.7
Dipel/+ Soil/Day0	6.0	13.8174	13.8381	20.7	0.9	96.2	7	30	23	23.3
Dipel/No Soil/Day14	0.1	13.7207	14.4377	717.0	24.7	-4.0	1	30	29	3.3
Dipel/No Soil/Day14	0.5	13.8633	14.4586	595.3	21.3	10.6	2	30	28	6.7
Dipel/No Soil/Day14	1.0	13.7447	14.1085	363.8	14.6	38.8	5	30	25	16.7
Dipel/No Soil/Day14	2.0	14.1620	14.4586	296.6	13.5	43.3	8	30	22	26.7
Dipel/No Soil/Day14	6.0	13.7176	13.7512	33.6	1.3	94.6	4	30	26	13.3
Dipel/No Soil/Day10	0.1	13.7468	13.9558	209.0	8.0	66.2	4	30	26	13.3
Dipel/No Soil/Day10	0.5	13.8668	13.9956	128.8	4.8	79.9	3	30	27	10.0
Dipel/No Soil/Day10	1.0	13.8679	14.0253	157.4	5.6	76.4	2	30	28	6.7
Dipel/No Soil/Day10	2.0	13.8061	14.0922	286.1	12.4	47.7	6	29	23	20.7
Dipel/No Soil/Day10	6.0	13.7585	13.8916	133.1	6.3	73.3	9	30	21	30.0
Dipel/+ Soil/Day10	0.1	14.1500	14.6061	456.1	16.3	31.5	2	30	28	6.7
Dipel/+ Soil/Day10	0.5	13.7160	13.8106	94.6	3.9	83.4	6	30	24	20.0
Dipel/+ Soil/Day10	1.0	13.7398	14.1604	420.6	16.8	29.3	6	31	25	19.4
Dipel/+ Soil/Day10	2.0	13.7217	14.1588	437.1	16.2	31.9	3	30	27	10.0
Dipel/+ Soil/Day10	6.0	13.9110	13.9750	64.0	3.2	86.5	9	29	20	31.0
Dipel/No Soil/Day5	0.1	13.7628	14.1653	402.5	14.4	39.5	2	30	28	6.7
Dipel/No Soil/Day5	0.5	13.7613	14.1870	425.7	16.4	31.1	4	30	26	13.3
Dipel/No Soil/Day5	1.0	13.7681	14.1144	346.3	12.4	48.0	2	30	28	6.7
Dipel/No Soil/Day5	2.0	13.7236	14.0583	334.7	13.4	43.7	5	30	25	16.7
Dipel/No Soil/Day5	6.0	13.7587	13.8343	75.6	4.0	83.3	10	29	19	34.5
Dipel/Fresh	0.1	13.7679	14.1933	425.4	14.2	40.4	0	30	30	0.0
Dipel/Fresh	0.5	13.8729	14.2405	367.6	13.6	42.7	3	30	27	10.0
Dipel/Fresh	1.0	13.8204	14.0476	227.2	8.4	64.7	3	30	27	10.0
Dipel/Fresh	2.0	13.7300	13.8201	90.1	4.3	82.0	9	30	21	30.0
Dipel/Fresh	6.0	13.6599	13.6807	20.8	1.0	95.6	8	28	20	28.6
Dipel + Soil/Fresh	0.1	13.8161	14.5021	686.0	22.9	3.8	0	30	30	0.0
Dipel + Soil/Fresh	0.5	14.1228	14.3348	212.0	8.5	64.3	5	30	25	16.7
Dipel + Soil/Fresh	1.0	13.7183	13.8275	109.2	4.7	80.0	7	30	23	23.3
Dipel + Soil/Fresh	2.0	13.6869	13.7821	95.2	3.3	86.2	1	30	29	3.3
Dipel + Soil/Fresh	6.0	13.7637	13.7911	27.4	1.2	94.8	7	29	22	24.1
Soil Fate Assay 6 - Started 11/04/98. Evaluated 11/10/98										
Control 1	0.0	13.7630	14.5846	821.6	27.4	2.6	0	30	30	0.0
Control 2	0.0	13.7204	14.5834	863.0	28.8	-2.3	0	30	30	0.0
Control 3	0.0	13.7459	15.9731	2227.2	28.2	-0.3	1	80	79	1.3

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Bacterial Cry1F/+ Soil/Day14	0.1	13.7551	14.5936	838.5	28.9	-2.8	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day14	0.5	13.7903	14.4432	652.9	21.8	22.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	1.0	13.6788	14.3686	689.8	23.0	18.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	2.0	13.9066	14.3940	487.4	16.2	42.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	6.0	13.7487	14.0979	349.2	11.6	58.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	0.1	13.7353	14.2822	546.9	18.2	35.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	0.5	13.7196	14.0071	287.5	9.6	65.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	1.0	13.7904	13.9036	113.2	3.9	86.1	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day0	2.0	14.1179	14.1946	76.7	2.7	90.3	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day0	6.0	13.7207	13.7317	11.0	0.6	97.7	13	30	17	43.3
Bacterial Cry1F/No Soil/Day7	0.1	13.7550	14.1291	374.1	12.5	55.6	0	30	30	0.0
Bacterial Cry1F/No Soil/Day7	0.5	13.7073	13.8035	96.2	3.2	88.6	0	30	30	0.0
Bacterial Cry1F/No Soil/Day7	1.0	13.8767	13.9226	45.9	1.8	93.7	4	30	26	13.3
Bacterial Cry1F/No Soil/Day7	2.0	13.7182	13.7464	28.2	1.4	95.0	10	30	20	33.3
Bacterial Cry1F/No Soil/Day7	6.0	13.6914	13.7089	17.5	1.8	93.8	20	30	10	66.7
Bacterial Cry1F/+ Soil/Day10	0.1	13.8654	14.7701	904.7	30.2	-7.3	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	0.5	13.7693	14.5126	743.3	24.8	11.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	1.0	13.7542	14.4171	662.9	22.9	18.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day10	2.0	13.8001	14.3093	509.2	17.0	39.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	6.0	13.7124	13.9852	272.8	9.1	67.7	0	30	30	0.0
Bacterial Cry1F/No Soil/Day10	0.1	13.7965	14.2220	425.5	14.7	47.8	1	30	29	3.3
Bacterial Cry1F/No Soil/Day10	0.5	13.7661	13.8962	130.1	4.5	84.0	1	30	29	3.3
Bacterial Cry1F/No Soil/Day10	1.0	13.7960	13.8321	36.1	1.4	95.1	4	30	26	13.3
Bacterial Cry1F/No Soil/Day10	2.0	13.8029	13.8468	43.9	2.1	92.6	9	30	21	30.0
Bacterial Cry1F/No Soil/Day10	6.0	13.6612	13.6671	5.9	0.5	98.3	17	29	12	58.6
Bacterial Cry1F/+ Soil/Day5	0.1	13.7246	14.4828	758.2	25.3	10.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	0.5	13.8426	14.5831	740.5	24.7	12.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	1.0	13.7100	14.2699	559.9	19.3	31.3	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day5	2.0	13.8737	14.2139	340.2	12.2	56.8	0	28	28	0.0
Bacterial Cry1F/+ Soil/Day5	6.0	13.8138	14.0015	187.7	6.5	77.0	1	30	29	3.3
Bacterial Cry1F/No Soil/Day28	0.1	13.8243	14.2406	416.3	13.9	50.6	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	0.5	13.8251	13.9533	128.2	4.7	83.1	2	29	27	6.9
Bacterial Cry1F/No Soil/Day28	1.0	13.7996	13.9231	123.5	4.1	85.4	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	2.0	13.7434	13.7965	53.1	2.2	92.1	6	30	24	20.0
Bacterial Cry1F/No Soil/Day28	6.0	13.7250	13.7274	2.4	0.6	97.9	25	29	4	86.2
Bacterial Cry1F/No Soil/Day0	0.1	13.8028	14.1742	371.4	12.4	56.0	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	0.5	13.7429	13.8812	138.3	4.6	83.6	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	1.0	14.1597	14.2362	76.5	2.9	89.5	4	30	26	13.3
Bacterial Cry1F/No Soil/Day0	2.0	13.7192	13.7380	18.8	0.9	96.8	9	30	21	30.0
Bacterial Cry1F/No Soil/Day0	6.0	13.7244	13.7295	5.1	0.4	98.5	18	30	12	60.0
Bacterial Cry1F/+ Soil/Day7	0.1	13.7313	14.6285	897.2	29.9	-6.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	0.5	13.7824	14.4539	671.5	23.2	17.6	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day7	1.0	13.7514	14.3404	589.0	20.3	27.8	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day7	2.0	14.1587	14.5328	374.1	12.5	55.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	6.0	13.7210	13.9307	209.7	7.0	75.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	0.1	13.7109	14.5835	872.6	29.1	-3.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	0.5	13.7337	14.6470	913.3	30.4	-8.3	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	1.0	13.8349	14.6469	812.0	27.1	3.7	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	2.0	13.7261	14.3240	597.9	20.6	26.7	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day28	6.0	13.8335	14.2093	375.8	12.5	55.4	0	30	30	0.0
Bacterial Cry1F/No Soil/Day3	0.1	13.8288	14.1766	347.8	11.6	58.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day3	0.5	13.7158	13.8249	109.1	3.9	86.1	2	30	28	6.7
Bacterial Cry1F/No Soil/Day3	1.0	13.7396	13.7991	59.5	2.8	89.9	9	30	21	30.0
Bacterial Cry1F/No Soil/Day3	2.0	13.7645	13.7710	6.5	0.4	98.7	12	30	18	40.0
Bacterial Cry1F/No Soil/Day3	6.0	13.7917	13.8037	12.0	1.5	94.7	22	30	8	73.3
Bacterial Cry1F/+ Soil/Day3	0.1	13.7166	14.4574	740.8	24.7	12.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.5	13.8409	14.4239	583.0	19.4	30.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	1.0	13.9240	14.2012	277.2	9.2	67.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	2.0	14.1268	14.3421	215.3	7.7	72.7	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day3	6.0	13.7679	13.8293	61.4	2.1	92.5	1	30	29	3.3
Bacterial Cry1F/No Soil/Day14	0.1	13.7398	14.1401	400.3	13.8	50.9	0	29	29	0.0
Bacterial Cry1F/No Soil/Day14	0.5	13.8542	13.9446	90.4	3.1	88.9	1	30	29	3.3

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Bacterial Cry1F/No Soil/Day14	1.0	13.7361	13.7778	41.7	2.2	92.2	11	30	19	36.7
Bacterial Cry1F/No Soil/Day14	2.0	13.9302	13.9589	28.7	1.7	94.0	13	30	17	43.3
Bacterial Cry1F/No Soil/Day14	6.0	13.7935	13.7984	4.9	0.4	98.5	17	29	12	58.6
Bacterial Cry1F/No Soil/Day5	0.1	13.7783	14.1193	341.0	12.6	55.1	3	30	27	10.0
Bacterial Cry1F/No Soil/Day5	0.5	13.7112	13.8240	112.8	4.0	85.7	1	29	28	3.4
Bacterial Cry1F/No Soil/Day5	1.0	13.9065	14.0149	108.4	4.2	85.2	4	30	26	13.3
Bacterial Cry1F/No Soil/Day5	2.0	13.7626	13.7856	23.0	1.3	95.5	12	30	18	40.0
Bacterial Cry1F/No Soil/Day5	6.0	13.7409	13.7451	4.2	0.7	97.5	24	30	6	80.0
Soil Only/Day14	0.1	13.9153	14.7978	882.5	29.4	-4.6	0	30	30	0.0
Soil Only/Day14	0.5	13.8312	14.6838	852.6	28.4	-1.1	0	30	30	0.0
Soil Only/Day14	1.0	13.7211	14.5674	846.3	28.2	-0.3	0	30	30	0.0
Soil Only/Day14	2.0	13.7808	14.5100	729.2	24.3	13.5	0	30	30	0.0
Soil Only/Day14	6.0	13.6999	14.5219	822.0	27.4	2.5	0	30	30	0.0
Bacterial Cry1F/Fresh	0.1	13.7493	14.1299	380.6	12.7	54.9	0	30	30	0.0
Bacterial Cry1F/Fresh	0.5	14.1209	14.2303	109.4	3.9	86.1	2	30	28	6.7
Bacterial Cry1F/Fresh	1.0	13.7675	13.7917	24.2	1.4	94.9	12	29	17	41.4
Bacterial Cry1F/Fresh	2.0	13.7678	13.7919	24.1	1.9	93.4	17	30	13	56.7
Bacterial Cry1F/Fresh	6.0	13.7593	13.7710	11.7	1.2	95.8	20	30	10	66.7
Bacterial Cry1F + Soil/Fresh	0.1	13.8705	14.3109	440.4	14.7	47.8	0	30	30	0.0
Bacterial Cry1F + Soil/Fresh	0.5	13.7146	13.8810	166.4	5.9	78.9	2	30	28	6.7
Bacterial Cry1F + Soil/Fresh	1.0	13.7371	13.7674	30.3	1.4	95.1	7	29	22	24.1
Bacterial Cry1F + Soil/Fresh	2.0	13.7848	13.7985	13.7	0.7	97.7	8	29	21	27.6
Bacterial Cry1F + Soil/Fresh	6.0	13.7969	13.8014	4.5	0.6	97.7	23	30	7	76.7
Soil Fate Assay 7 - Started 11/06/98, Evaluated 11/12/98										
Control 1	0.0	13.7681	14.7214	953.3	31.8	4.4	0	30	30	0.0
Control 2	0.0	13.7550	14.8181	1063.1	35.4	-6.6	0	30	30	0.0
Control 3	0.0	13.7539	16.4836	2729.7	32.5	2.2	0	84	84	0.0
Bacterial Cry1F/+ Soil/Day28	0.1	13.7473	14.6312	883.9	29.5	11.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	0.5	13.7203	14.6141	893.8	29.8	10.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	1.0	13.8610	14.6885	827.5	27.6	17.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day28	2.0	13.7335	14.4433	709.8	24.5	26.4	0	29	29	0.0
Bacterial Cry1F/+ Soil/Day28	6.0	14.1588	14.5263	367.5	12.3	63.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	0.1	13.7543	14.6857	931.4	31.0	6.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	0.5	13.7782	14.5560	777.8	25.9	22.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	1.0	13.6903	14.4642	773.9	25.8	22.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	2.0	14.1225	14.7403	617.8	20.6	38.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day14	6.0	13.7960	14.1211	325.1	10.8	67.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	0.1	13.7780	14.6466	868.6	29.0	12.9	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	0.5	13.7672	14.0919	324.7	11.2	66.3	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day7	1.0	13.7563	13.9735	217.2	7.8	76.7	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day7	2.0	13.8320	13.9238	91.8	3.1	90.8	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day7	6.0	14.1618	14.4304	268.6	9.0	73.1	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	0.1	14.1454	14.7355	590.1	19.7	40.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	0.5	13.7648	13.9659	201.1	6.7	79.8	0	30	30	0.0
Bacterial Cry1F/No Soil/Day28	1.0	13.7243	13.8305	106.2	4.6	86.1	7	30	23	23.3
Bacterial Cry1F/No Soil/Day28	2.0	13.7295	13.7987	69.2	3.8	88.4	12	30	18	40.0
Bacterial Cry1F/No Soil/Day28	6.0	13.7068	13.7170	10.2	0.9	97.4	18	30	12	60.0
Bacterial Cry1F/No Soil/Day0	0.1	13.8506	14.0703	219.7	7.3	78.0	0	30	30	0.0
Bacterial Cry1F/No Soil/Day0	0.5	13.7181	13.8816	163.5	5.6	83.0	1	30	29	3.3
Bacterial Cry1F/No Soil/Day0	1.0	13.8200	13.8859	65.9	2.6	92.1	5	30	25	16.7
Bacterial Cry1F/No Soil/Day0	2.0	14.1544	14.1632	8.8	0.5	98.5	12	30	18	40.0
Bacterial Cry1F/No Soil/Day0	6.0	13.8303	13.8445	14.2	1.3	96.1	19	30	11	63.3
Bacterial Cry1F/+ Soil/Day5	0.1	13.9080	14.8214	913.4	30.4	8.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	0.5	13.8174	14.4690	651.6	21.7	34.7	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	1.0	13.7554	14.3280	572.6	19.1	42.6	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	2.0	13.7205	14.0184	297.9	9.9	70.1	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day5	6.0	13.8341	13.9886	154.5	5.2	84.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.1	13.7425	14.6252	882.7	29.4	11.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	0.5	13.7880	14.3724	584.4	19.5	41.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	1.0	13.9223	14.4777	555.4	19.2	42.4	1	30	29	3.3
Bacterial Cry1F/+ Soil/Day3	2.0	14.1241	14.3682	244.1	8.1	75.5	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day3	6.0	13.7260	13.8611	135.1	4.8	85.5	2	30	28	6.7

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Number Total	Number Alive	Percent Control
Bacterial Cry1F/+ Soil/Day10	0.1	13.8032	14.7438	940.6	31.4	5.7	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	0.5	13.6812	14.4971	815.9	27.2	18.2	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	1.0	13.7744	14.4782	703.8	23.5	29.4	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	2.0	13.7931	14.5325	739.4	24.6	25.8	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day10	6.0	13.7602	14.0961	335.9	11.2	66.3	0	30	30	0.0
Bacterial Cry1F/No Soil/Day10	0.1	13.7851	14.2851	500.0	17.2	48.1	1	30	29	3.3
Bacterial Cry1F/No Soil/Day10	0.5	13.7614	13.9548	193.4	6.9	79.2	2	30	28	6.7
Bacterial Cry1F/No Soil/Day10	1.0	13.7151	13.7584	43.3	1.9	94.3	7	30	23	23.3
Bacterial Cry1F/No Soil/Day10	2.0	13.7729	13.8264	53.5	2.3	93.0	7	30	23	23.3
Bacterial Cry1F/No Soil/Day10	6.0	14.1236	14.1256	2.0	0.5	98.5	26	30	4	86.7
Bacterial Cry1F/+ Soil/Day0	0.1	13.7247	14.4553	730.6	24.4	26.7	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	0.5	13.7981	14.0873	289.2	9.6	71.0	0	30	30	0.0
Bacterial Cry1F/+ Soil/Day0	1.0	13.7459	13.9394	193.5	6.9	79.2	2	30	28	6.7
Bacterial Cry1F/+ Soil/Day0	2.0	13.7187	13.8061	87.4	3.2	90.3	3	30	27	10.0
Bacterial Cry1F/+ Soil/Day0	6.0	13.8489	13.8946	45.7	2.1	93.8	8	30	22	26.7
Bacterial Cry1F/No Soil/Day7	0.1	13.7562	14.1600	403.8	13.5	59.5	0	30	30	0.0
Bacterial Cry1F/No Soil/Day7	0.5	13.8711	14.0475	176.4	6.5	80.3	2	29	27	6.9
Bacterial Cry1F/No Soil/Day7	1.0	13.7616	13.8496	88.0	3.5	89.4	5	30	25	16.7
Bacterial Cry1F/No Soil/Day7	2.0	13.6991	13.7275	28.4	1.6	95.3	12	30	18	40.0
Bacterial Cry1F/No Soil/Day7	6.0	13.7352	13.7361	0.9	0.3	99.1	27	30	3	90.0
Bacterial Cry1F/No Soil/Day5	0.1	13.8556	14.3028	447.2	14.9	55.1	0	30	30	0.0
Bacterial Cry1F/No Soil/Day5	0.5	13.7615	13.9384	176.9	6.3	81.0	2	30	28	6.7
Bacterial Cry1F/No Soil/Day5	1.0	13.8303	13.8732	42.9	1.8	94.6	6	30	24	20.0
Bacterial Cry1F/No Soil/Day5	2.0	13.7955	13.8333	37.8	2.5	92.4	15	30	15	50.0
Bacterial Cry1F/No Soil/Day5	6.0	13.7576	13.7645	6.9	0.8	97.7	21	30	9	70.0
Bacterial Cry1F/No Soil/Day3	0.1	13.8320	14.4332	601.2	20.0	39.7	0	30	30	0.0
Bacterial Cry1F/No Soil/Day3	0.5	13.7598	13.9481	188.3	6.7	79.8	2	30	28	6.7
Bacterial Cry1F/No Soil/Day3	1.0	13.8668	13.9366	69.8	3.2	90.5	8	30	22	26.7
Bacterial Cry1F/No Soil/Day3	2.0	13.8553	13.8899	34.6	2.2	93.5	14	30	16	46.7
Bacterial Cry1F/No Soil/Day3	6.0	13.7198	13.7302	10.4	1.0	96.9	20	30	10	66.7
Bacterial Cry1F/No Soil/Day14	0.1	13.7575	14.4469	689.4	23.0	30.9	0	30	30	0.0
Bacterial Cry1F/No Soil/Day14	0.5	13.7663	14.1004	334.1	11.9	64.1	1	29	28	3.4
Bacterial Cry1F/No Soil/Day14	1.0	13.7049	13.8463	141.4	7.1	78.7	9	29	20	31.0
Bacterial Cry1F/No Soil/Day14	2.0	13.7642	13.7919	27.7	1.5	95.6	11	30	19	36.7
Bacterial Cry1F/No Soil/Day14	6.0	13.8022	13.8191	16.9	1.9	94.4	21	30	9	70.0
Soil Only/Day28	0.1	14.1167	15.3386	1221.9	40.7	-22.5	0	30	30	0.0
Soil Only/Day28	0.5	14.1234	15.2974	1174.0	39.1	-17.7	0	30	30	0.0
Soil Only/Day28	1.0	13.7629	14.8999	1137.0	37.9	-14.0	0	30	30	0.0
Soil Only/Day28	2.0	13.7159	14.7700	1054.1	35.1	-5.7	0	30	30	0.0
Soil Only/Day28	6.0	13.7201	14.7917	1071.6	35.7	-7.5	0	30	30	0.0
Bacterial Cry1F/Fresh	0.1	13.6889	14.1584	469.5	16.2	51.3	1	30	29	3.3
Bacterial Cry1F/Fresh	0.5	13.8159	13.9819	166.0	5.5	83.4	0	30	30	0.0
Bacterial Cry1F/Fresh	1.0	13.7208	13.8272	106.4	4.6	86.1	7	30	23	23.3
Bacterial Cry1F/Fresh	2.0	13.7697	13.8247	55.0	2.5	92.5	8	30	22	26.7
Bacterial Cry1F/Fresh	6.0	13.7421	13.7442	2.1	0.3	99.2	22	30	8	73.3
Bacterial Cry1F + Soil/Fresh	0.1	13.7338	14.1553	421.5	14.1	57.7	0	30	30	0.0
Bacterial Cry1F + Soil/Fresh	0.5	13.7588	13.9400	181.2	6.5	80.5	2	30	28	6.7
Bacterial Cry1F + Soil/Fresh	1.0	13.8943	13.9358	41.5	2.1	93.8	9	29	20	31.0
Bacterial Cry1F + Soil/Fresh	2.0	13.7577	13.8040	46.3	3.6	89.3	17	30	13	56.7
Bacterial Cry1F + Soil/Fresh	6.0	14.1635	14.1659	2.4	0.6	98.2	25	29	4	86.2
Soil Fate Assay 8 - Started 11/13/98, Evaluated 11/19/98										
Control 1	0.0	13.7947	14.6944	899.7	31.0	2.5	1	30	29	3.3
Control 2	0.0	13.8892	14.8704	981.2	32.7	-2.8	0	30	30	0.0
Control 3	0.0	13.7809	15.4629	1682.0	31.7	0.3	2	55	53	3.6
Dipel/No Soil/Day3	0.1	13.8938	14.6165	722.7	25.8	18.9	1	29	28	3.4
Dipel/No Soil/Day3	0.5	13.7020	14.2193	517.3	18.5	41.9	2	30	28	6.7
Dipel/No Soil/Day3	1.0	13.7944	14.2621	467.7	16.7	47.5	2	30	28	6.7
Dipel/No Soil/Day3	2.0	13.6996	13.8804	180.8	7.2	77.3	5	30	25	16.7
Dipel/No Soil/Day3	6.0	13.8254	13.8988	73.4	3.2	90.0	7	30	23	23.3
Dipel/+ Soil/Day3	0.1	13.8483	14.6286	780.3	26.9	15.4	1	30	29	3.3
Dipel/+ Soil/Day3	0.5	13.7780	14.3544	576.4	20.6	35.3	2	30	28	6.7
Dipel/+ Soil/Day3	1.0	13.7678	14.0641	296.3	10.6	66.7	2	30	28	6.7

Treatment	Rate	Tube weight (g)	Tube weight + larvae (g)	Total Larval weight (mg)	Weight / Larva (mg)	% Growth Inhibition	Number Dead	Total	Number Alive	Percent Control
Dipel/+ Soil/Day3	2.0	13.8381	14.0346	196.5	7.0	77.9	2	30	28	6.7
Dipel/+ Soil/Day3	6.0	13.7503	13.8155	65.2	3.1	90.2	8	29	21	27.6
Dipel/No Soil/Day10	0.1	13.7922	14.5191	726.9	25.1	21.2	0	29	29	0.0
Dipel/No Soil/Day10	0.5	13.7548	14.3361	581.3	21.5	32.3	3	30	27	10.0
Dipel/No Soil/Day10	1.0	13.7023	14.3110	608.7	22.5	29.2	3	30	27	10.0
Dipel/No Soil/Day10	2.0	13.7828	14.1831	400.3	19.1	40.1	9	30	21	30.0
Dipel/No Soil/Day10	6.0	13.7405	13.9163	175.8	8.4	73.7	9	30	21	30.0
Dipel/+ Soil/Day28	0.1	14.1130	15.0097	896.7	29.9	6.1	0	30	30	0.0
Dipel/+ Soil/Day28	0.5	13.8118	14.5009	689.1	24.6	22.7	2	30	28	6.7
Dipel/+ Soil/Day28	1.0	13.7014	14.3195	618.1	21.3	33.0	1	30	29	3.3
Dipel/+ Soil/Day28	2.0	13.6973	14.1883	491.0	18.2	42.9	3	30	27	10.0
Dipel/+ Soil/Day28	6.0	14.1159	14.3323	216.4	8.0	74.8	3	30	27	10.0
Dipel/No Soil/Day28	0.1	13.6939	14.5241	830.2	29.7	6.8	2	30	28	6.7
Dipel/No Soil/Day28	0.5	13.6973	14.4225	725.2	25.9	18.6	2	30	28	6.7
Dipel/No Soil/Day28	1.0	14.1701	14.7207	550.6	21.2	33.5	4	30	26	13.3
Dipel/No Soil/Day28	2.0	13.8337	14.2815	447.8	17.9	43.7	5	30	25	16.7
Dipel/No Soil/Day28	6.0	13.2830	13.3842	101.2	6.7	78.8	15	30	15	50.0
Soil Only/Day0	0.1	13.2896	14.1545	864.9	28.8	9.4	0	30	30	0.0
Soil Only/Day0	0.5	13.2920	14.1250	833.0	28.7	9.7	1	30	29	3.3
Soil Only/Day0	1.0	13.4176	14.2870	869.4	30.0	5.8	0	29	29	0.0
Soil Only/Day0	2.0	13.2954	14.1118	816.4	27.2	14.5	0	30	30	0.0
Soil Only/Day0	6.0	13.4347	14.2605	825.8	28.5	10.5	1	30	29	3.3
Dipel/+ Soil/Day10	0.1	13.3687	14.2400	871.3	30.0	5.6	1	30	29	3.3
Dipel/+ Soil/Day10	0.5	13.3330	14.0327	699.7	24.1	24.2	1	30	29	3.3
Dipel/+ Soil/Day10	1.0	13.3327	13.8143	481.6	18.5	41.8	4	30	26	13.3
Dipel/+ Soil/Day10	2.0	13.2901	13.6496	359.5	13.8	56.5	4	30	26	13.3
Dipel/+ Soil/Day10	6.0	13.4349	13.5614	126.5	5.5	82.7	7	30	23	23.3
Dipel/Fresh	0.1	13.2927	14.0814	788.7	27.2	14.5	0	29	29	0.0
Dipel/Fresh	0.5	13.3811	13.8211	440.0	15.7	50.6	2	30	28	6.7
Dipel/Fresh	1.0	13.3478	13.5821	234.3	8.7	72.7	2	29	27	6.9
Dipel/Fresh	2.0	13.4406	13.6317	191.1	6.6	79.3	1	30	29	3.3
Dipel/Fresh	6.0	13.3332	13.4013	68.1	2.5	92.1	3	30	27	10.0
Dipel + Soil/Fresh	0.1	13.3547	13.9915	636.8	22.0	31.0	1	30	29	3.3
Dipel + Soil/Fresh	0.5	13.3804	13.6965	316.1	10.5	66.9	0	30	30	0.0
Dipel + Soil/Fresh	1.0	13.3745	13.7080	333.5	11.1	65.1	0	30	30	0.0
Dipel + Soil/Fresh	2.0	13.2261	13.3669	140.8	4.9	84.7	1	30	29	3.3
Dipel + Soil/Fresh	6.0	13.3403	13.3874	47.1	1.6	94.9	0	29	29	0.0
Bacterial Cry1F/Fresh	0.1	13.3912	13.8324	441.2	14.7	53.8	0	30	30	0.0
Bacterial Cry1F/Fresh	0.5	13.3731	13.5155	142.4	4.9	84.6	1	30	29	3.3
Bacterial Cry1F/Fresh	1.0	13.3181	13.4049	86.8	3.2	89.9	3	30	27	10.0
Bacterial Cry1F/Fresh	2.0	13.4359	13.4816	45.7	2.3	92.8	10	30	20	33.3
Bacterial Cry1F/Fresh	6.0	13.7159	13.7221	6.2	0.5	98.4	18	30	12	60.0
Bacterial Cry1F + Soil/Fresh	0.1	14.1329	14.5845	451.6	15.1	52.7	0	30	30	0.0
Bacterial Cry1F + Soil/Fresh	0.5	13.8025	13.9514	148.9	5.0	84.4	0	30	30	0.0
Bacterial Cry1F + Soil/Fresh	1.0	13.8485	13.9258	77.3	2.6	91.9	0	30	30	0.0
Bacterial Cry1F + Soil/Fresh	2.0	13.7591	13.7822	23.1	1.1	96.5	8	29	21	27.6
Bacterial Cry1F + Soil/Fresh	6.0	13.7216	13.7441	22.5	1.3	95.8	13	30	17	43.3

**APPENDIX D**

**SAS CODE USED FOR GENERATING ED<sub>50</sub> VALUES**

```
data RawData;
  infile "saswghts.csv" dlm = "," firstobs=2;
  input ID $ @@;
      do dose=0,0.1, 0.5,1.0,2.0,6.0;
        input wt @@; output;
      end;
  proc sort; by ID dose;

proc means data=RawData noprint;
  var wt;
  by ID dose;
  output out=Main(drop=_freq_ _type_) mean=AveWt;
  run;
  proc print; run;

proc nlin data=Main;
  parameters W0=50 EC50=.5 beta = 1;
  model AveWt = W0/(1+(dose/EC50)**beta);
  by ID;
run;
```

**APPENDIX E**

**RESULTS AND METHODS FOR CALCULATING DT<sub>50</sub> VALUES  
FOR CRY1F AND DIPEL IN SOIL**

CALCULATIONS FOR DT<sub>50</sub>

To calculate a DT<sub>50</sub> (time required for the sample to decay to one-half its original strength) the natural logarithm of the reciprocal of the ED<sub>50</sub> at each point was calculated and plotted against time. These values are presented in the Table below. The loss of activity was linear between 0 and 14 days; after this time loss of activity was no longer linear so only Days 0 to 14 were included in the DT<sub>50</sub> analysis.

Time (Days)	ED <sub>50</sub> (ml of test solution)	1/ED <sub>50</sub>	ln(1/ED <sub>50</sub> )
0	0.17	5.88	1.77
3	0.77	1.30	0.26
5	1.04	0.96	-0.04
7	1.02	0.98	-0.02
10	2.43	0.41	-0.89
14	3.26	0.31	-1.18
28	3.87	0.26	-1.35

Using a hand held Hewlett Packard 15C calculator, a regression line was calculated having a slope of -0.221. The equation for calculating a half life (assuming first order kinetics) is:

$$t_{1/2} = 0.693/\text{slope}$$

Solving for  $t_{1/2}$ , gives a half life of 3.13 days

**APPENDIX F**

**COPY OF GLP SOIL CHARACTERIZATION REPORT**



Highway 15  
P.O. Box 510  
Northwood, ND 58267  
(701) 587-6010  
FAX (701) 587-6013

AGVISE Soil Characterization Report

Submitting firm = RICERCA INC.  
Protocol or Study No = 7569-98-0080-AC  
Sample ID. = U OF IL SOIL 0-15CM  
Trial ID. = NA  
Date Received = 7-7-98  
Date Reported = 07-13-1998

AGVISE Lab No 98- 1581  
Percent Sand 21  
Percent Silt 58  
Percent Clay 21  
USDA Textural Class (hydrometer method) Silt Loam  
Bulk Density (disturbed) gm/cc 1.07  
Cation Exchange Capacity (meq/100 g) 28.7  
% Moisture at 1/3 Bar 32.4  
Percent Organic Matter 6.1  
pH 7.0

Base Saturation Data		
Cation	Percent	ppm
Calcium	64.5	3700
Magnesium	20.6	710
Sodium	0.7	48
Potassium	2.4	271
Hydrogen	11.7	34

These tests were completed in compliance of 40 CFR Part 160.

*Robert Deutsch*  
Robert Deutsch  
Soil Scientist

7/13/98  
Date

VERIFIED COPY  
*JD* 12-1-98  
Initial Date

Agricultural Testing

DATE: 12/15/98  
SIGNATURE: *[Signature]*

**APPENDIX G**

**MOISTURE LOSS DATA**

Eunit	Day 0	Day 3	Day 5	Day 7	Day 10	Day 14	Day 28	Loss to Date
Moisture Tube Rep1	18.0309	18.0050	17.9888	17.9744	17.9543	17.9167	17.7825	0.2484
Moisture Tube Rep2	18.0114	17.9887	17.9747	17.9630	17.9469	17.9164	17.807	0.2044
Moisture Tube Rep3	18.0899	18.0696	18.0579	18.0451	18.0296	17.9995	17.8937	0.1962
Moisture Tube Rep4	17.9560	17.9299	17.9130	17.8987	17.8795	17.844	17.7154	0.2406
Moisture Tube Rep5	18.0031	17.9815	17.9685	17.9573	17.9413	17.9106	17.8013	0.2018
Moisture Tube Rep6	18.0611	18.0420	18.0306	18.0201	18.0072	17.9795	17.8846	0.1765

**APPENDIX H**

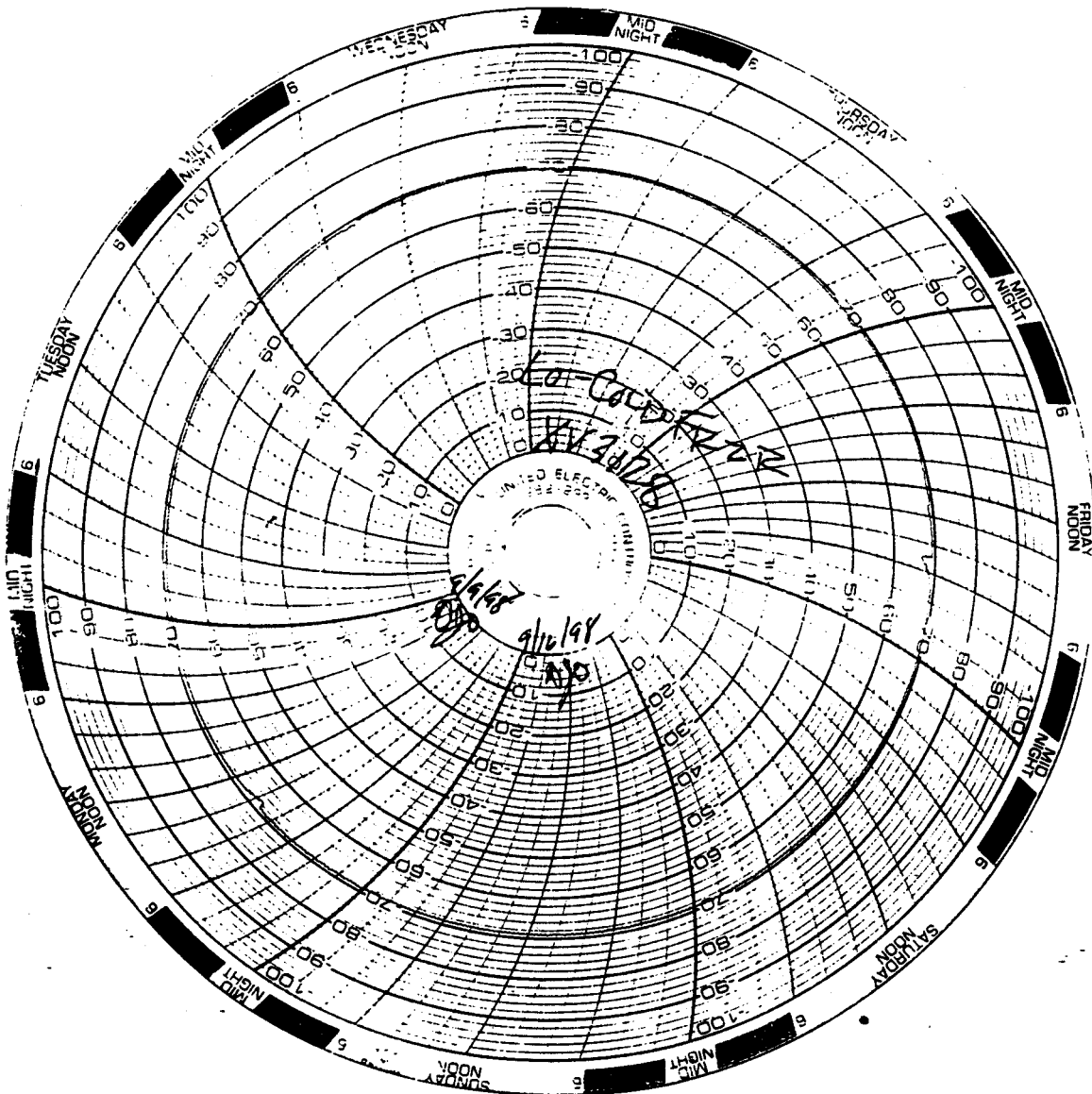
**CERTIFIED COPIES OF TEMPERATURE/RELATIVITY RECORDS  
FOR LO-COLD FREEZER, INCUBATION CHAMBER (LABELED  
C1), FORMA SCIENTIFIC INCUBATOR AND RELEVANT PAGES  
FROM INCUBATOR LOGBOOK DOCUMENTING PEN  
MALFUNCTION.**

**Temperature chart records for Lo-Cold freezer in which samples were kept until bioassay.**

7569-98-0080-AC

EXACT COPY

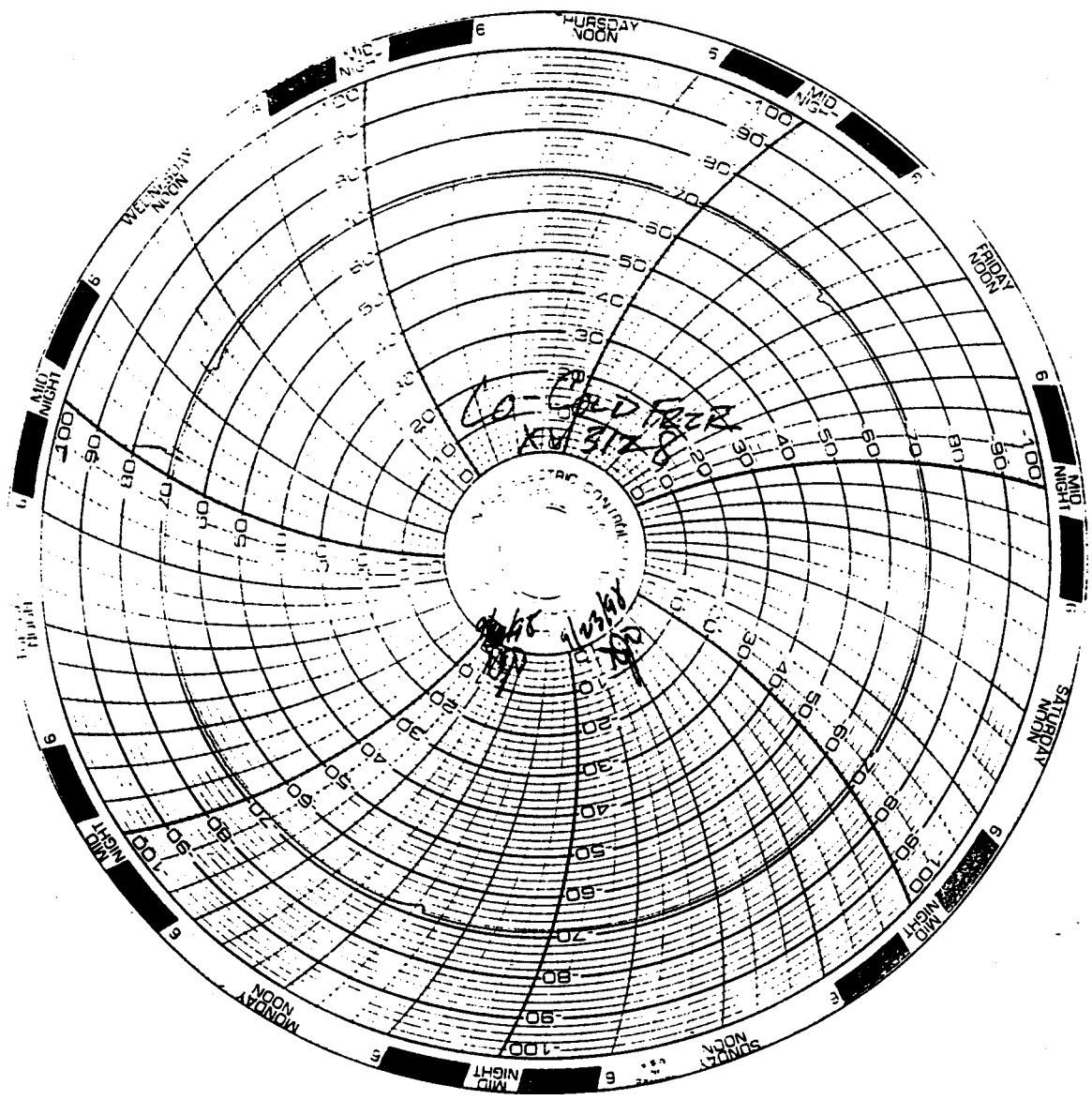
By: WRA Date: 12/15/98



7569-98-0080-AC

EXACT COPY

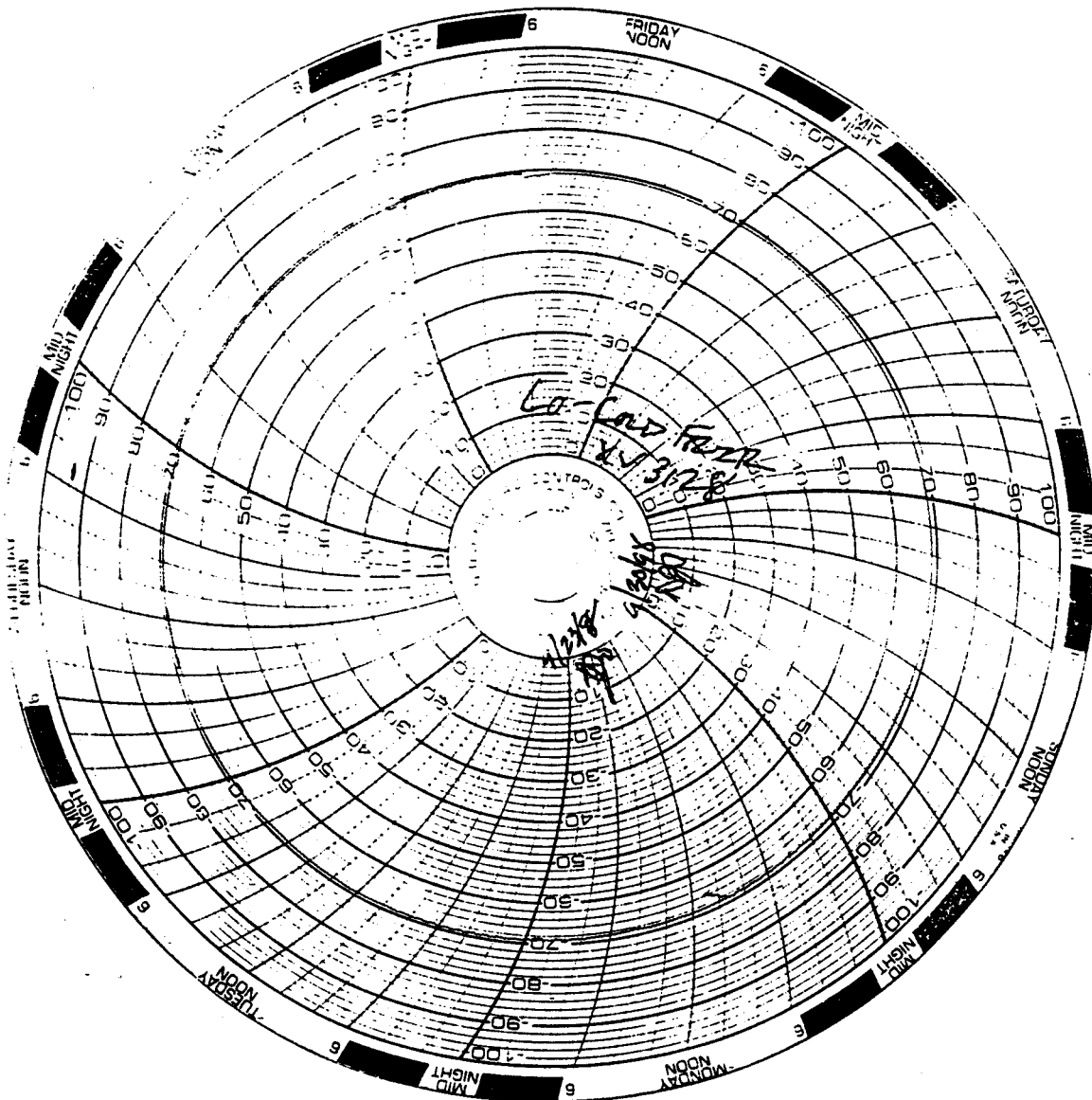
BY: UNM DATE: 12/15/88



7569-98-0080-AC

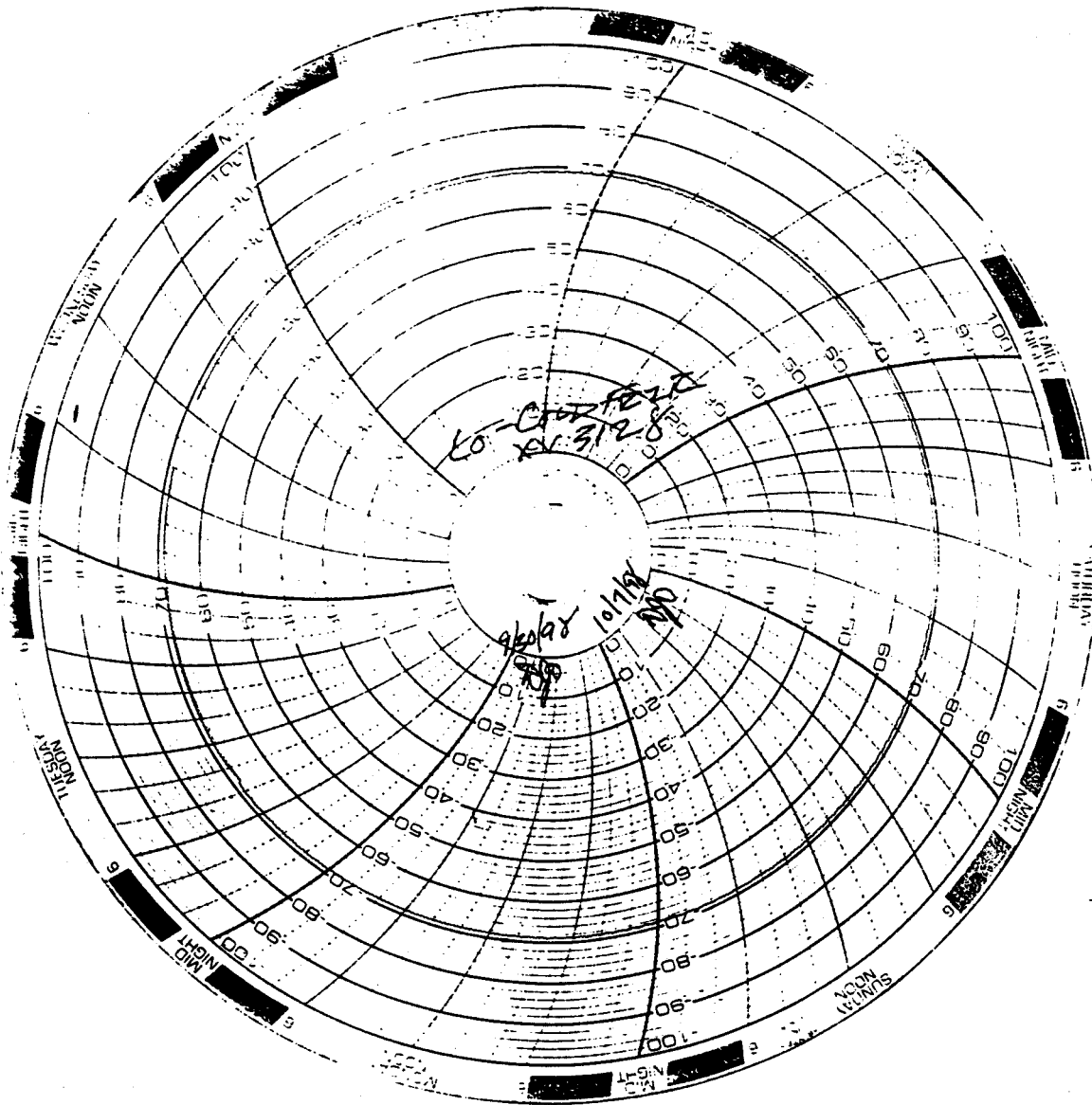
EXACT COPY

By: LMH Date: 12/15/98



7569-98-0080-AC

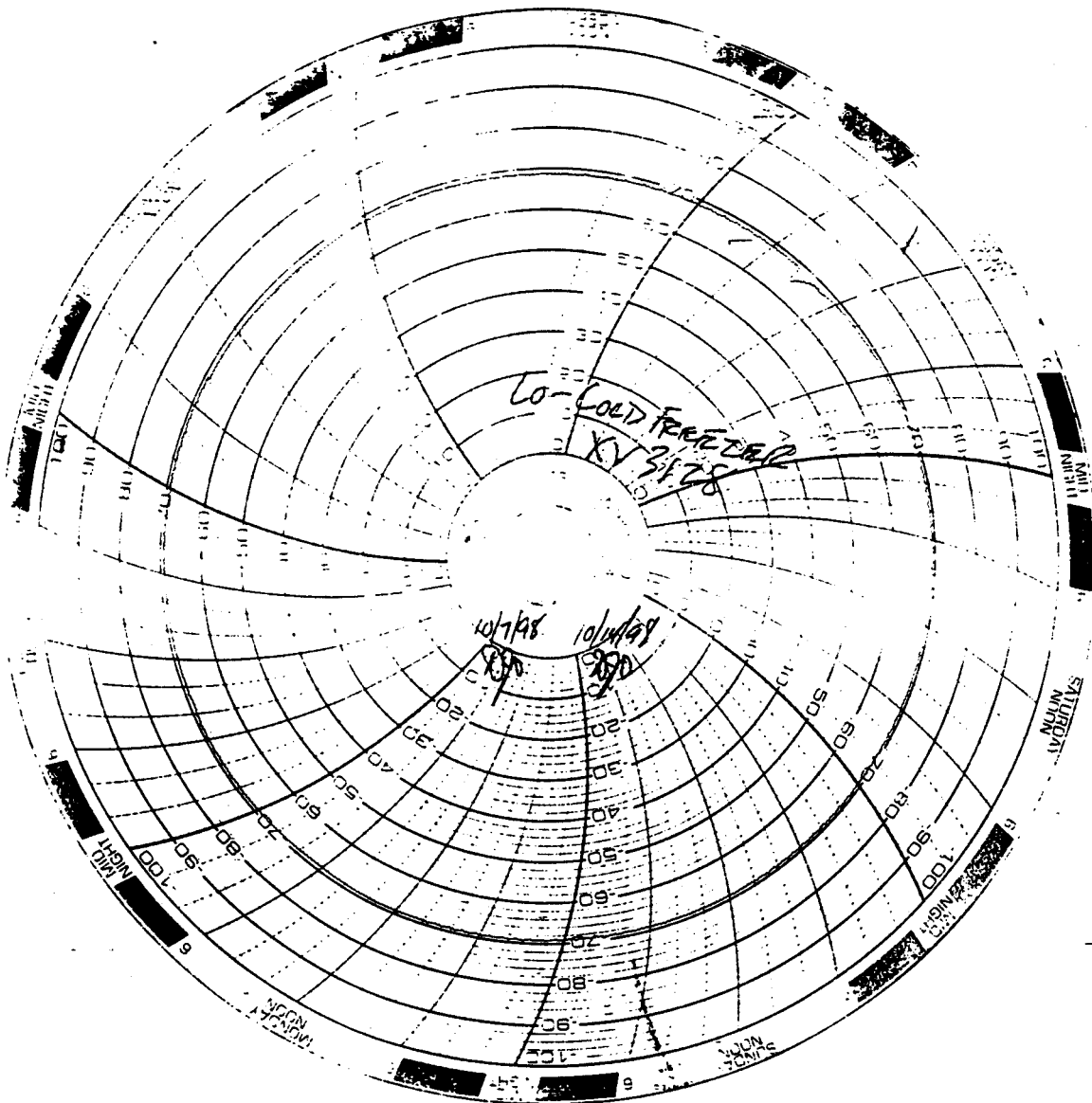
WZLH 12/11/98



7569-98-0080-AC

EXACT COPY

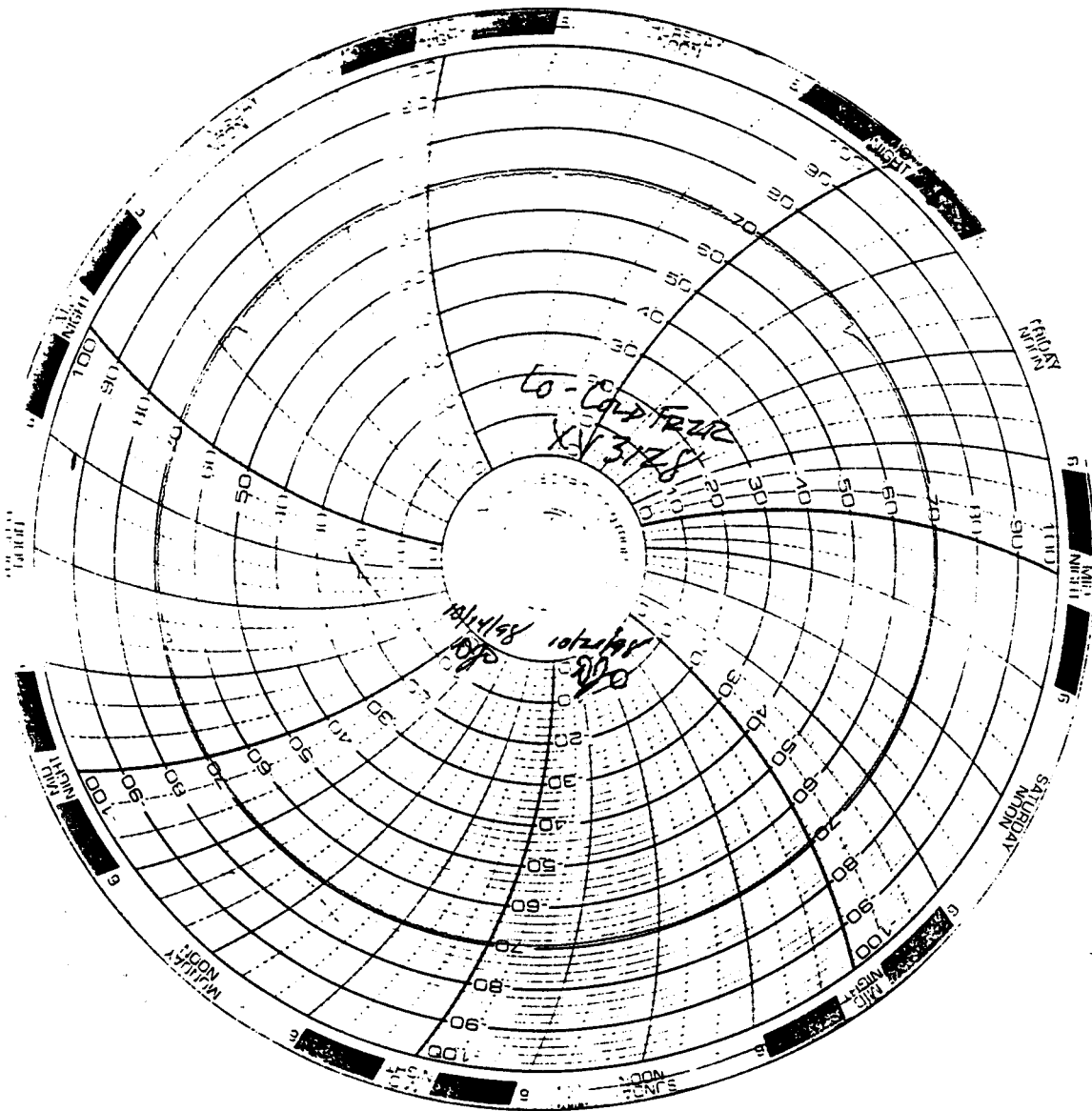
By: WMA Date: 12/15/88



7569-98-0080-AC

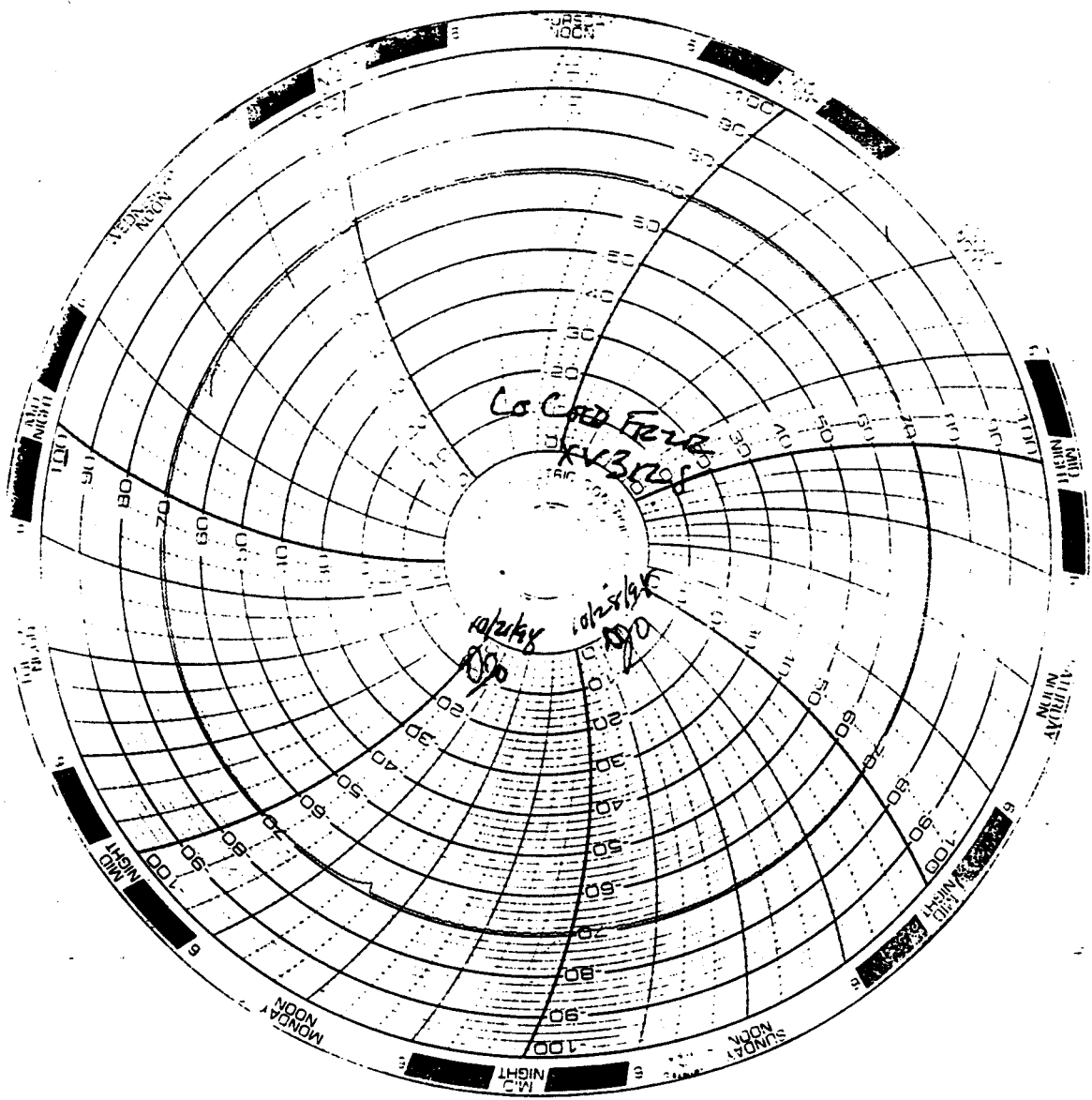
EXACT COPY

Bv: WVA Date: 12/15/98



7569-98-0080-AC

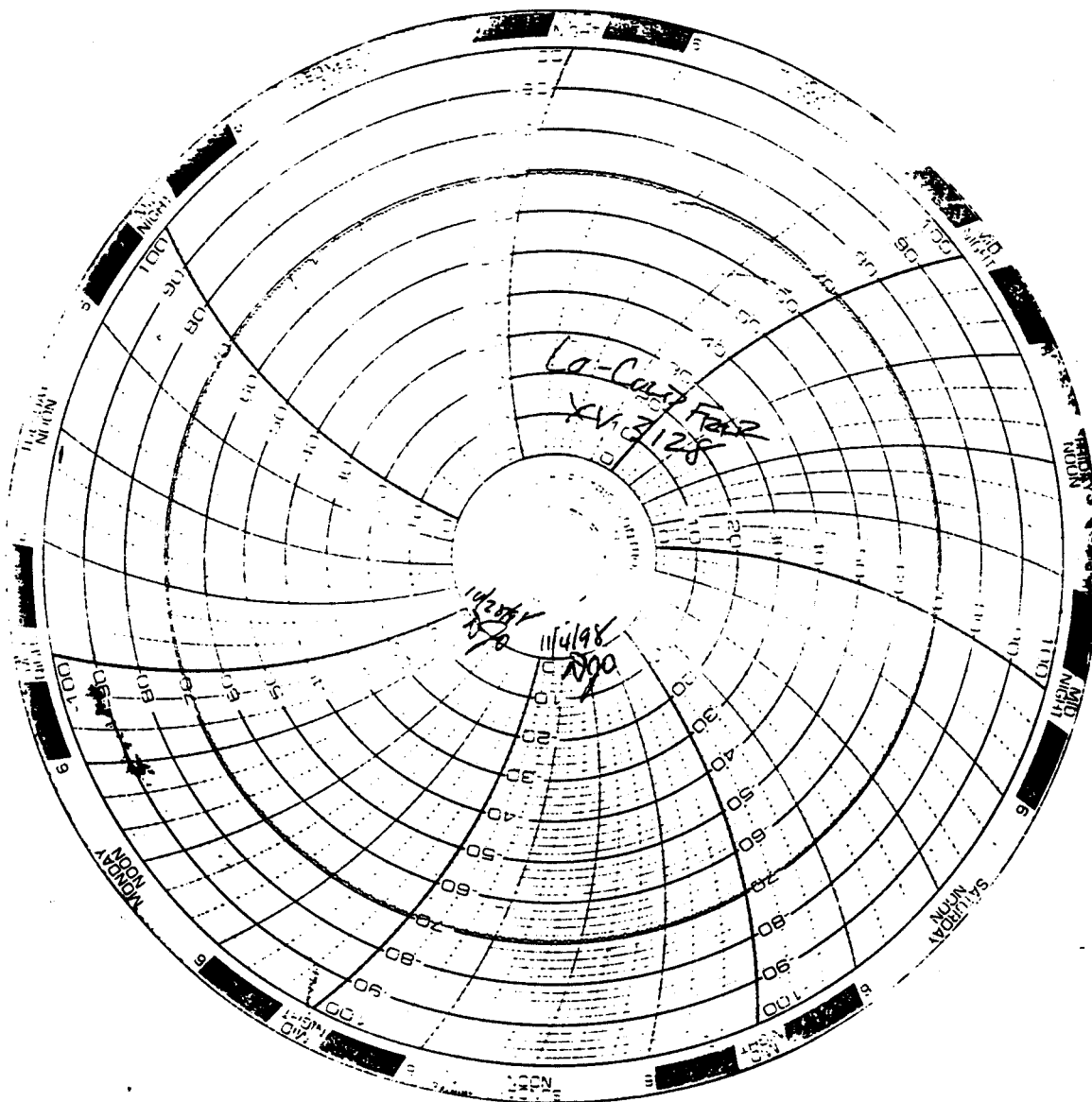
Site: WMA Date: 12/15/98



7569-98-0080-AC

EXACT COPY

Ev: WMT Date: 12/15/88

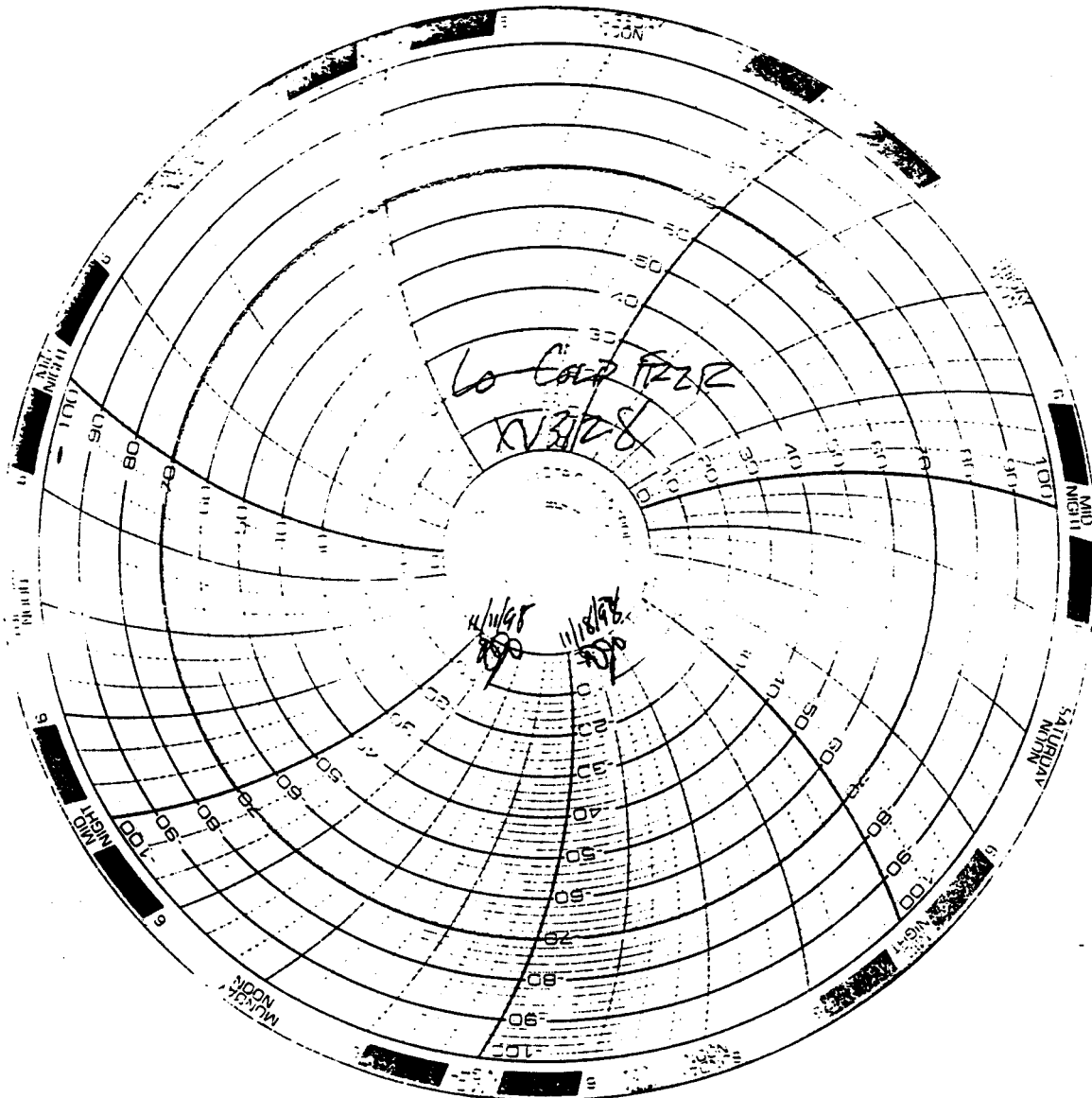




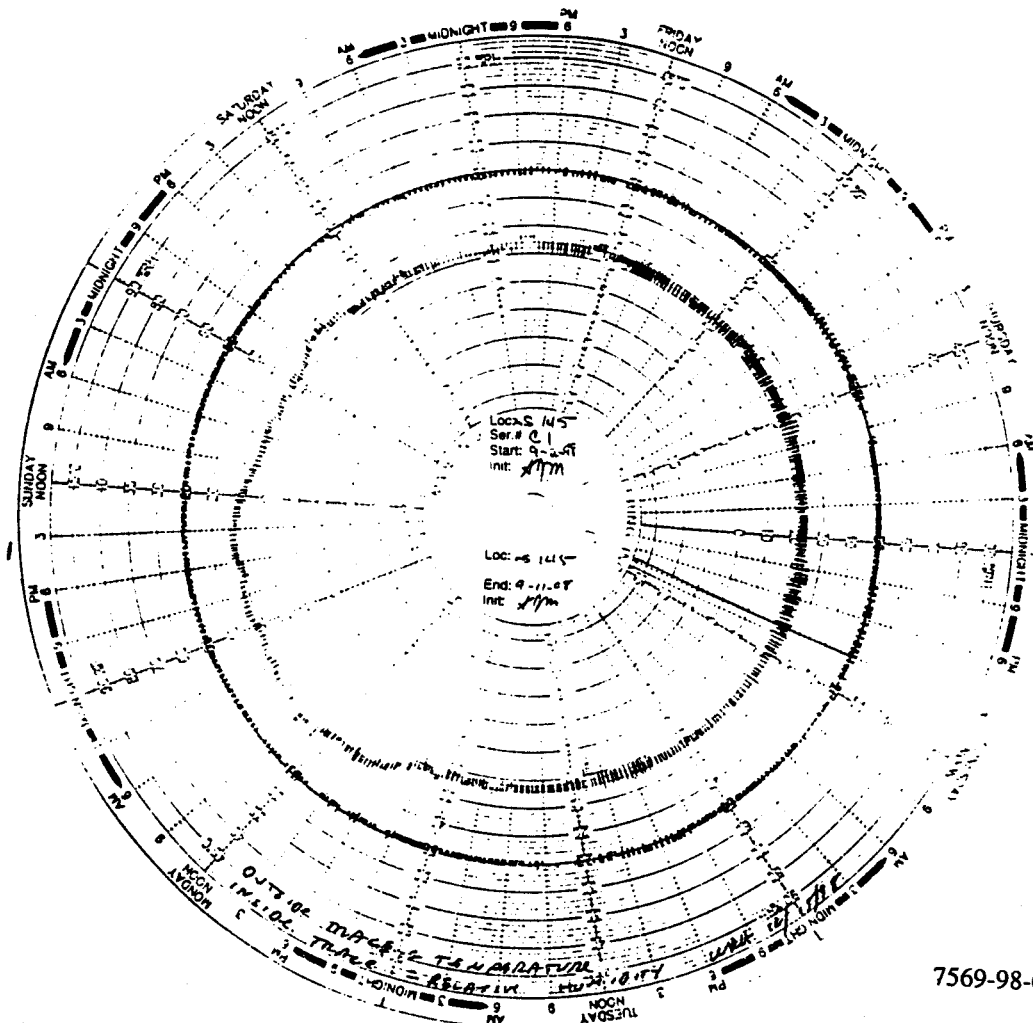
7569-98-0080-AC

EXACT COPY

By: WWT Date: 2/15/98

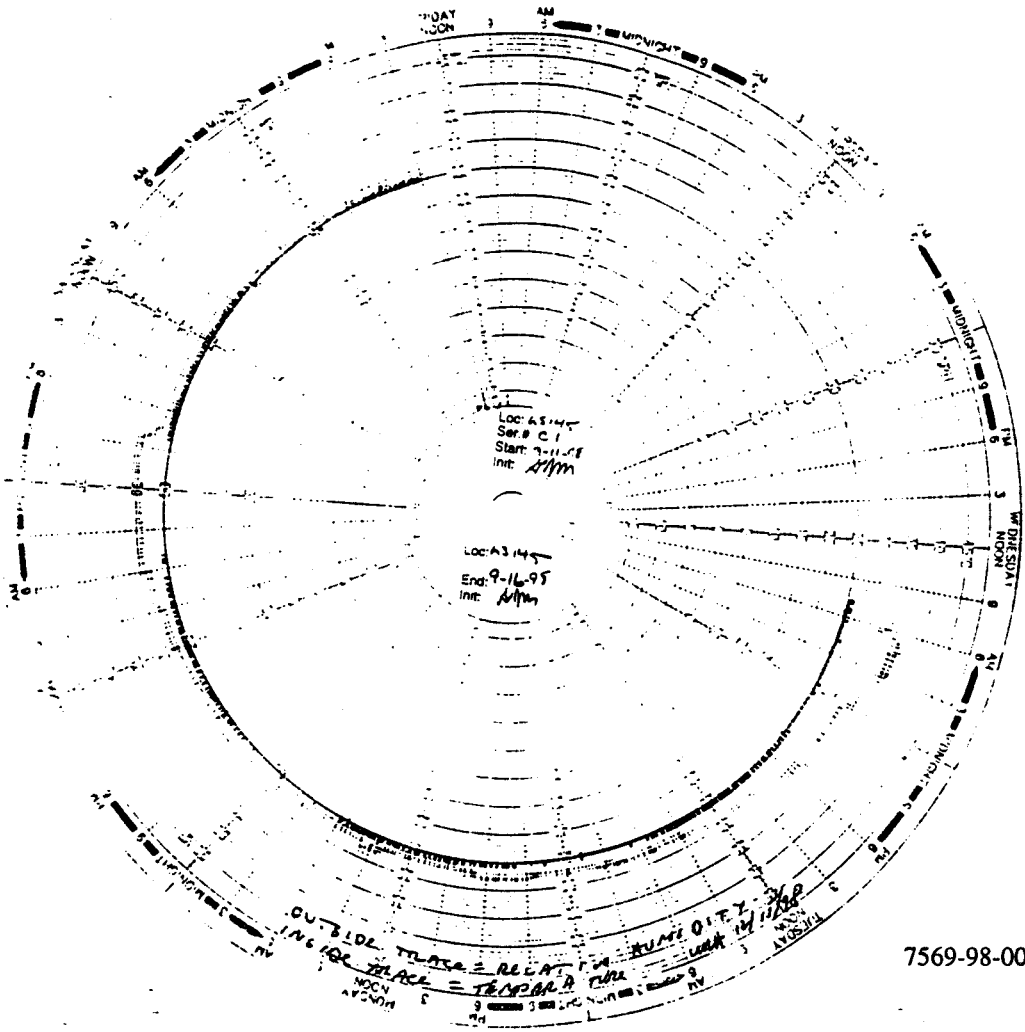


**Temperature/Relative humidity charts for Percival C1 chamber in which experiment was conducted.**



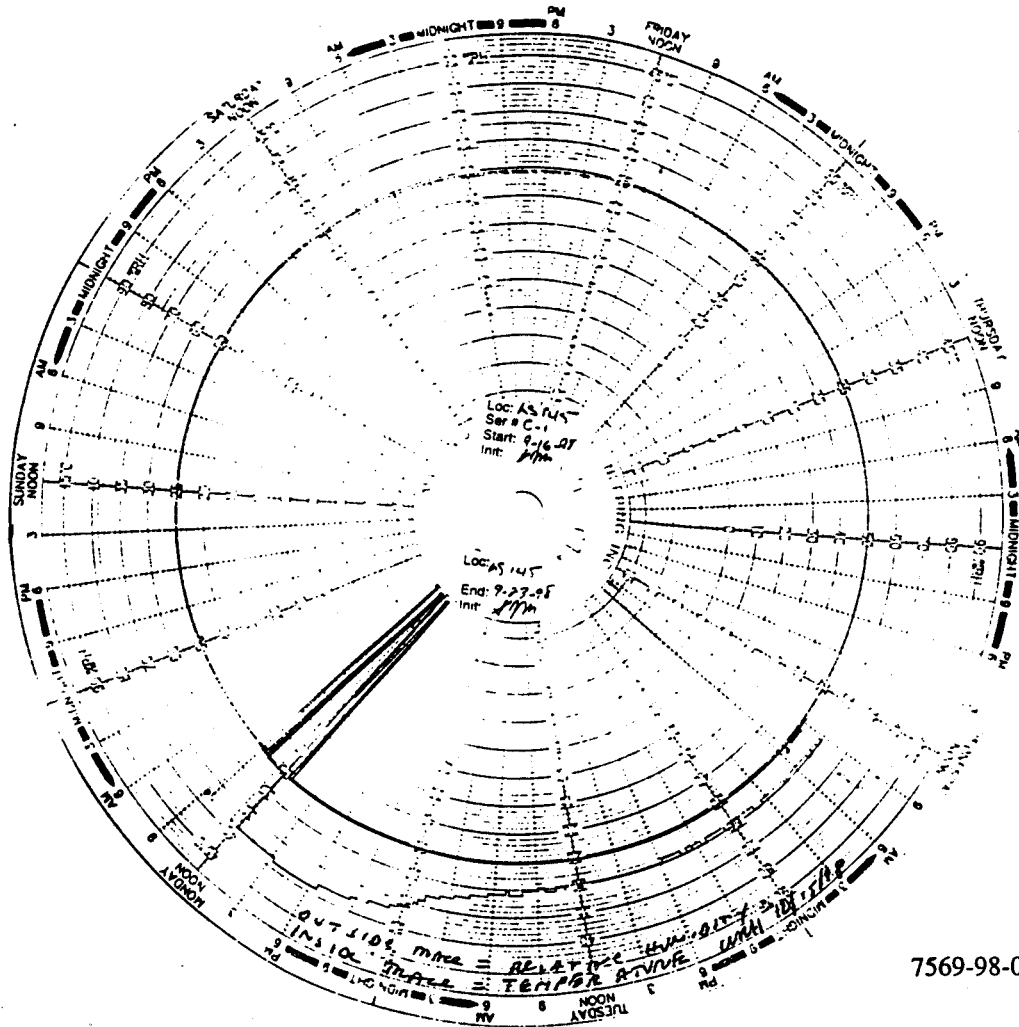
7569-98-0080-AC

THIS IS AN EXACT REPRODUCTION OF THE ORIGINAL DOCUMENT  
DATE: 12/15/98  
REPRODUCED BY: *unhanded*



7569-98-0080-AC

DATE: 12/15/98  
SIGNATURE: *W. Wallis*

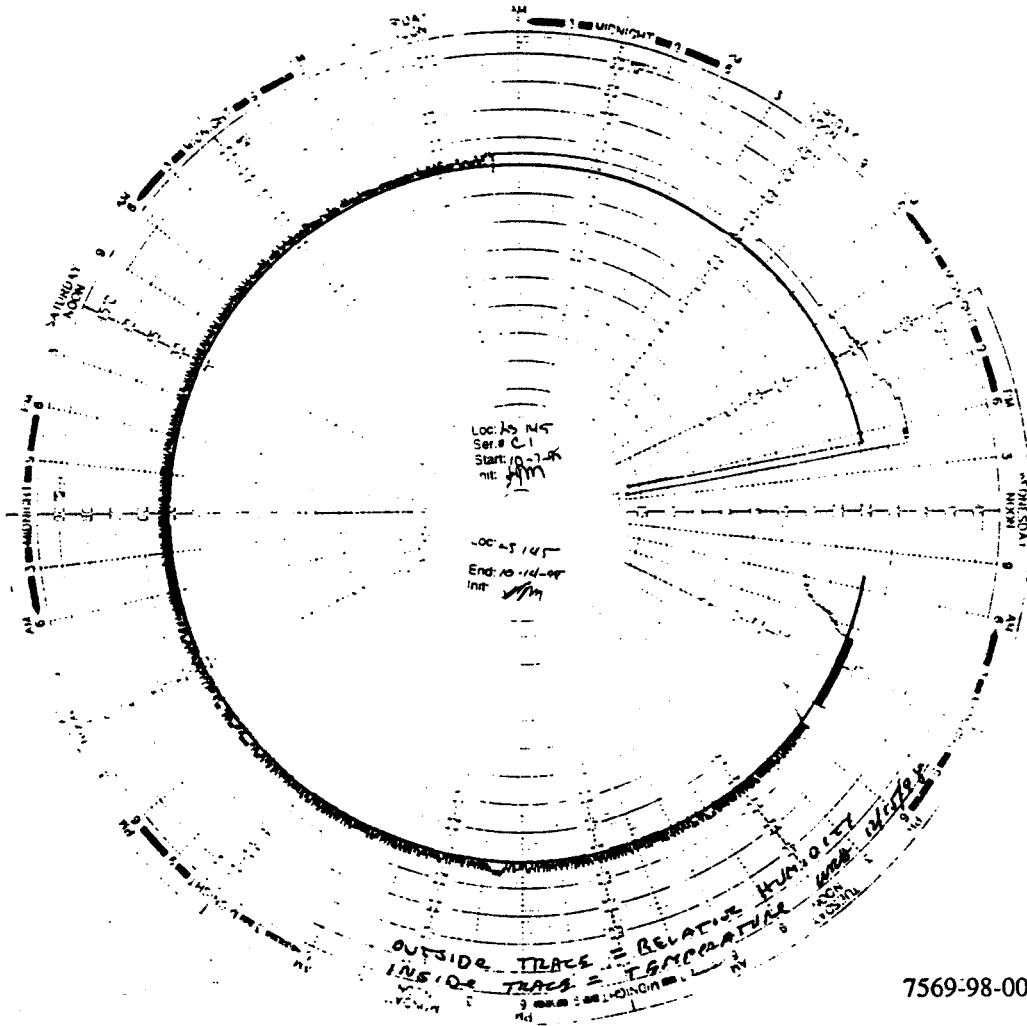


7569-98-0080-AC

THIS IS AN EXACT REPRODUCTION OF THE ORIGINAL DOCUMENT  
DATE: 12/15/98  
SIGNATURE: [Handwritten Signature]







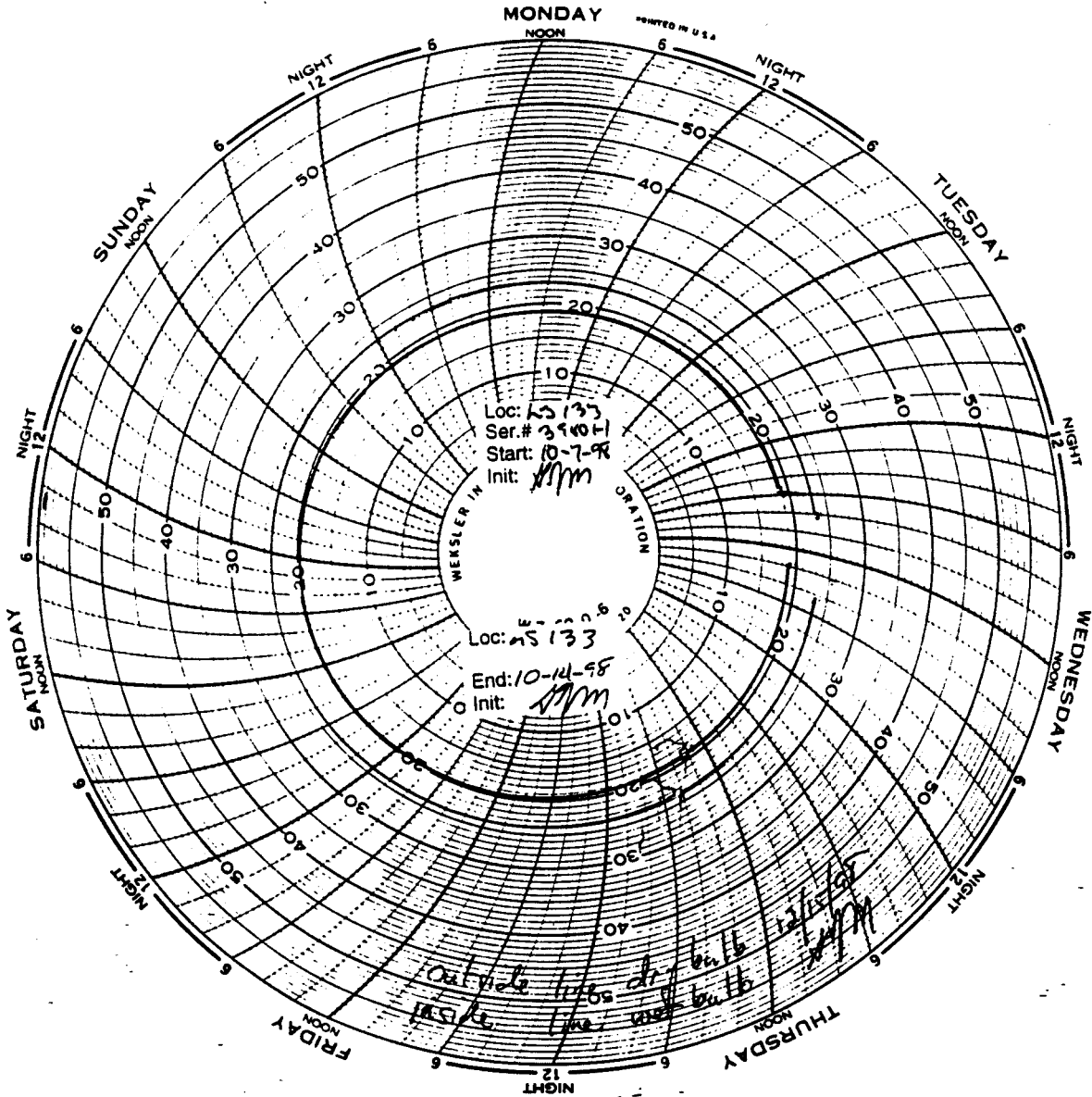
THIS IS A COPY OF THE ORIGINAL DOCUMENT  
DATE: 12/15/98  
BY: L.M. Hallie

**Temperature/Relative humidity charts for Forma Scientific Incubator in  
LS133 in which bioassay trays were maintained.**

7569-98-0080-AC

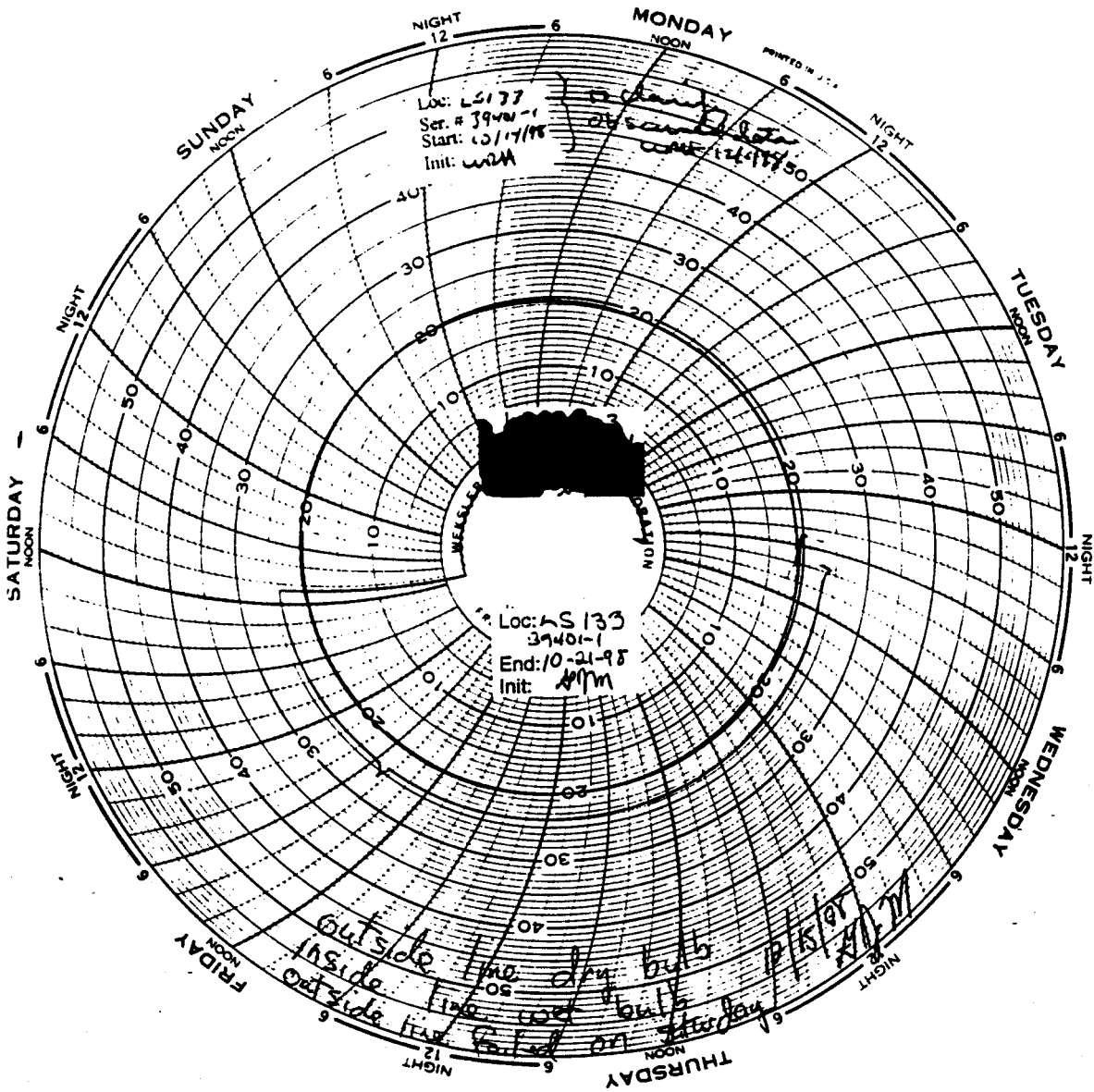
EXACT COPY

By: wnit Date: 12/15/98



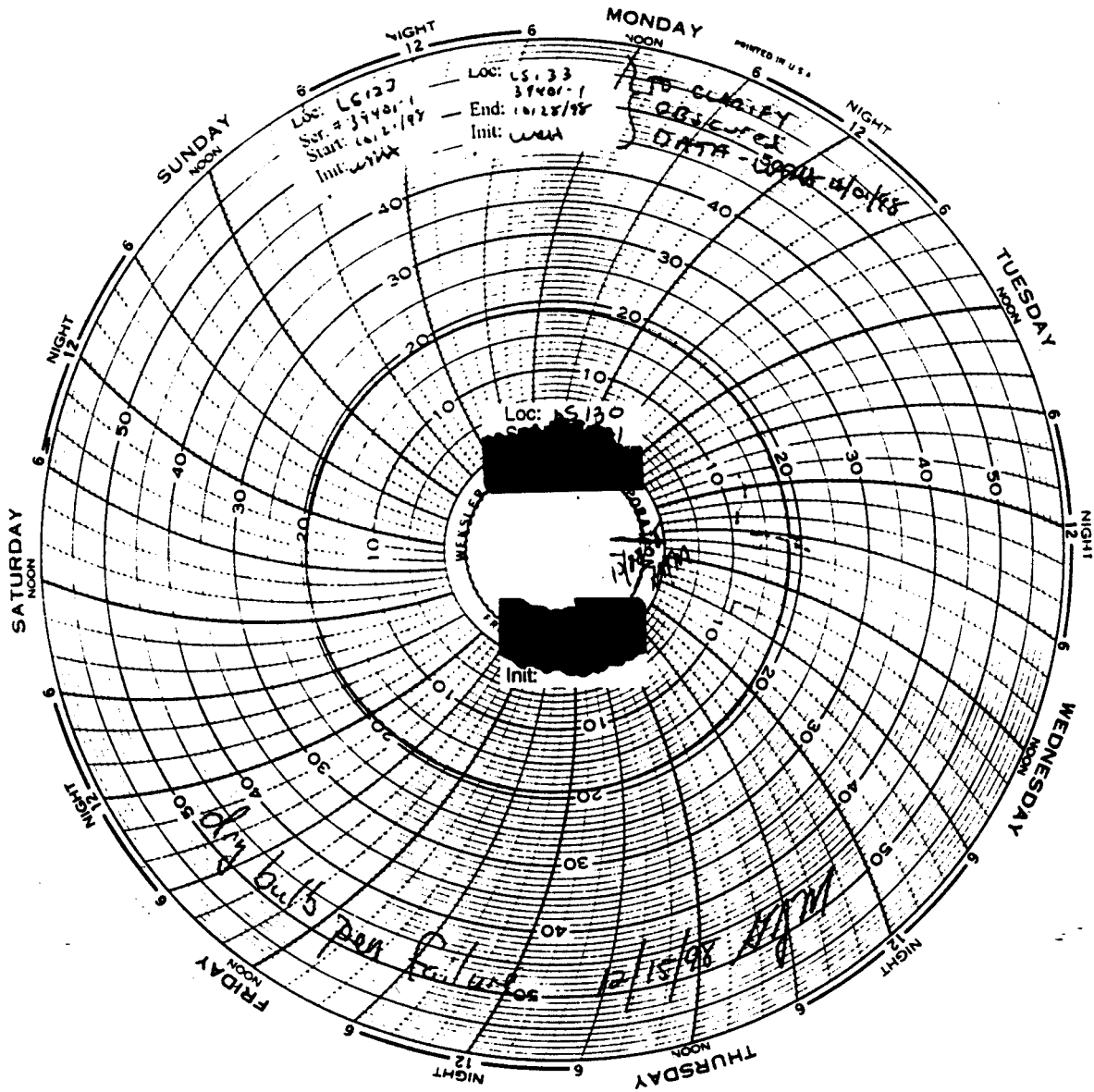
1569-98-0080-AC

*WMA* 12/15/98



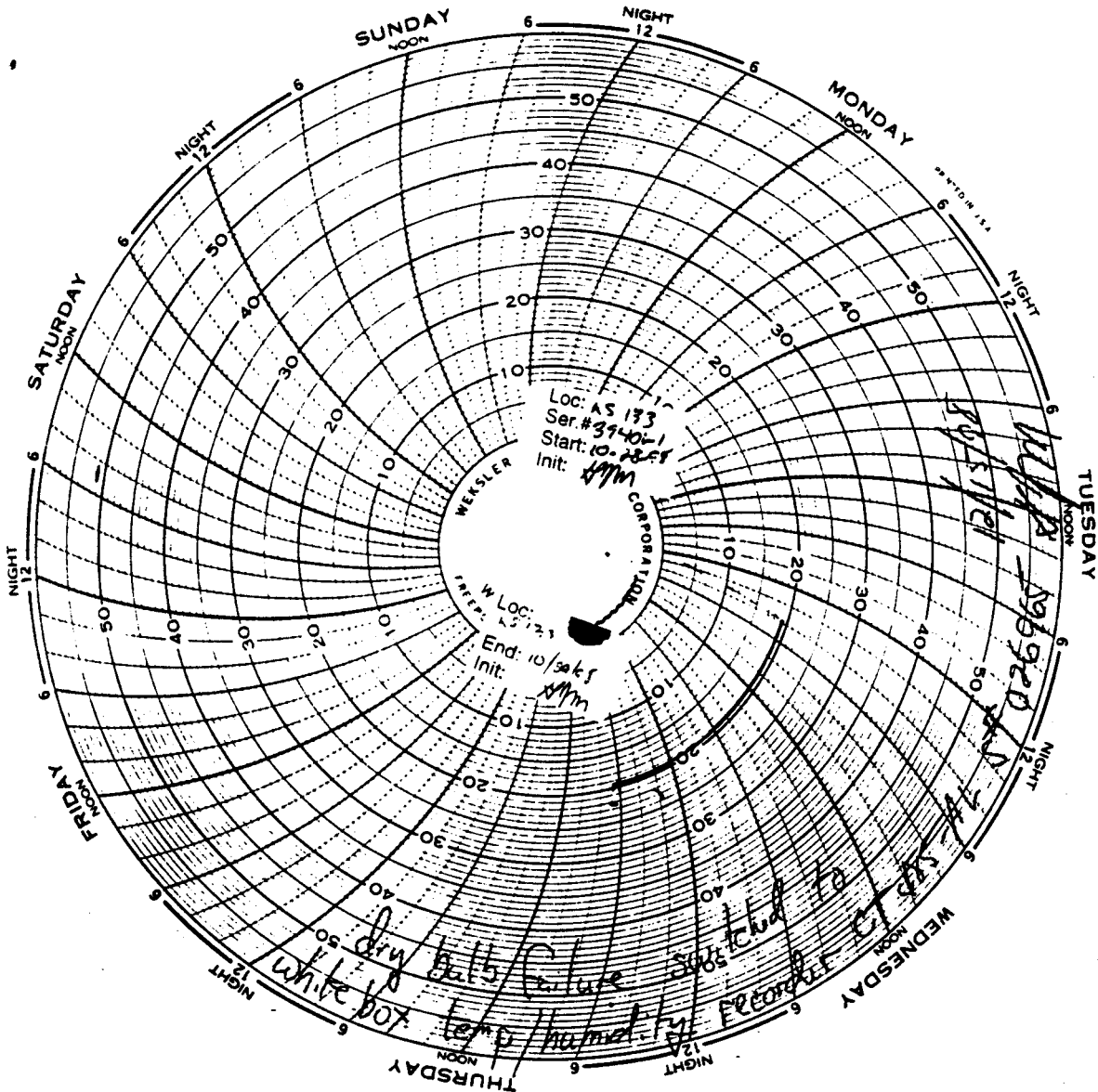
7569-98-0080-AC

Site: WMA Date: 12/15/98

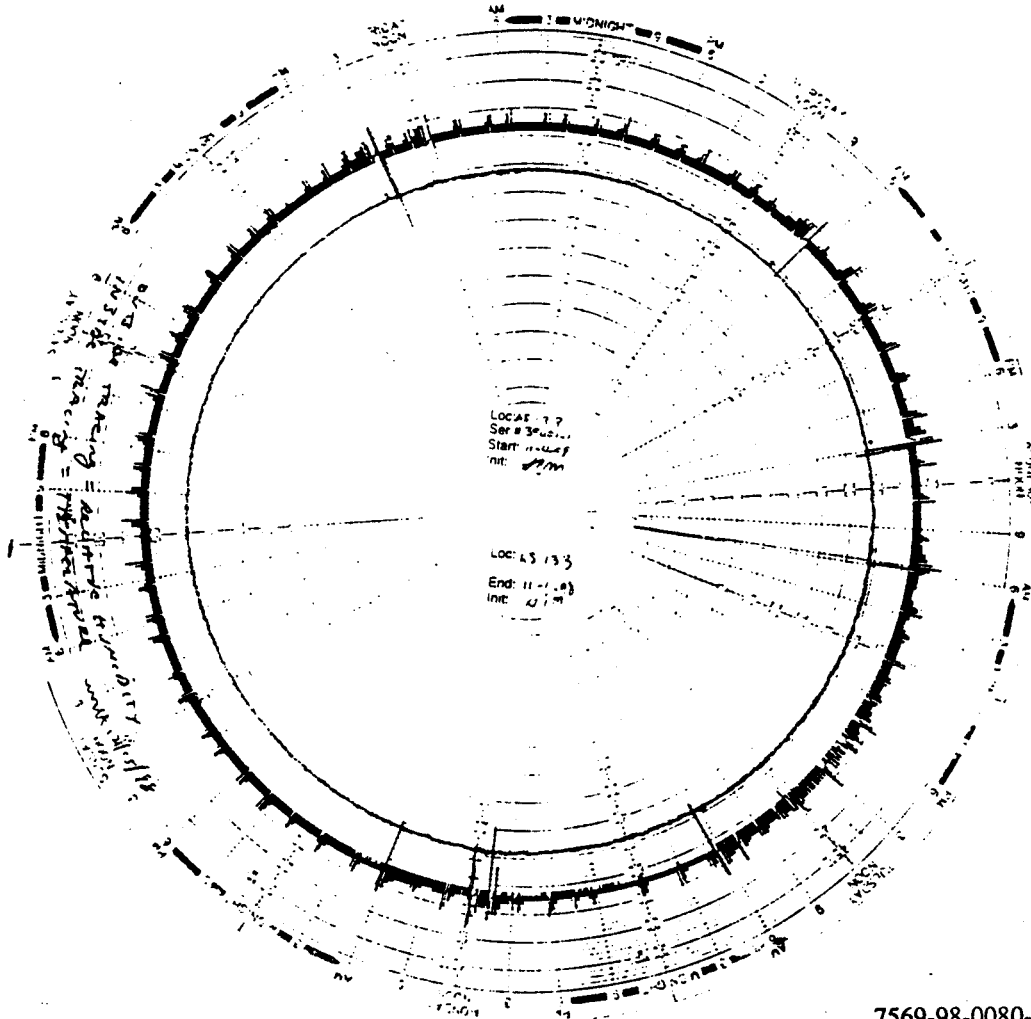


7569-98-0080-AC

Unit 12/15/98





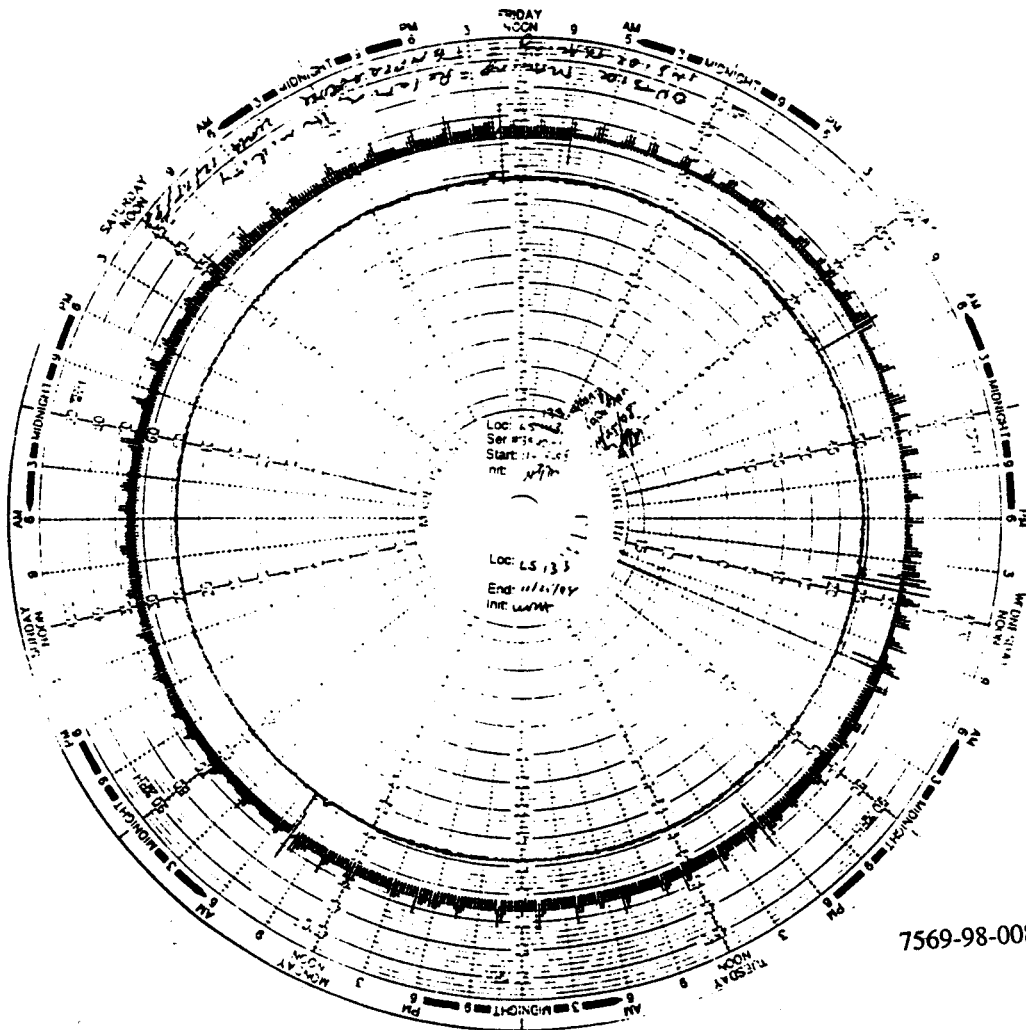


7569-98-0080-AC

12/15/98

UM Halliday





7569-98-0080-AC

12/15/95  
L. Wallis

Logbook Cover and relevant pages documenting failure of dry bulb recording pen.

Ricerca, Inc.

# LOGBOOK 00320

Page 1 of 50

	PAGE(S)
EQUIPMENT DESCRIPTION	1
PERSON RESPONSIBLE FOR EQUIPMENT	1
SOP REFERENCE	1
PERIPHERAL COMPONENTS & SOFTWARE	2
EQUIPMENT USERS	4
LOGBOOK RECORDS	6 - 50

## EQUIPMENT DESCRIPTION

<small>Name / Manufacturer / Model</small>	<i>Form Scientific Incubator 3940</i>
<small>Identifying Number (e.g., serial number, or version number)</small>	<i>39401-1</i>
<small>Installation date</small>	<i>1985</i>

Person responsible for equipment

*P. Halliday*

<small>SOP (exclude version no.)</small>	<small>PROTOCOL (for qualifications)</small>
<i>17-C017</i>	
<small>Logbook Use Dates</small>	<small>FROM: <i>7/23/98</i> TO:</small>

In the event of equipment failure or unusual event, record the following in the logbook:

- the nature of the failure or event
- how and when the event was discovered
- action taken in response to failure or event.

5/98

EXACT COPY  
 By: *C. NB*  
 Date: *12/1/98*  
 7569-98-0080-AC

Logbook Records

Page 8 of 58

Date		R/NR/Q	Initials
7-5-98	25	R	MM
9-16-98	24	R	MM
9-21-98	24	R	MM
9-22-98	24	R	MM
9-23-98	24	R	MM
10-6-98	24	R	MM
10-7-98	24	R	MM
10-8-98	24	R	MM
10-9-98	24	R	MM
10-13-98	24	R	MM
10-14-98	24	R	MM
10-19-98	24	R	MM
10-30-98	25	R	MM
10-30-98	out bulb is malfunctioning temp and humidity sensor (replaced 11/15/98)		MM

R = Routine; NR = Nonroutine (as defined by equipment SOP); NR entry may be made by service personnel; Q = Qualification



Logbook Records

Page 9 of 58

Date		R/NR/Q	Initials
10-21-98	24	R	MM
10-26-98	25	R	MM
10-29-98	24	R	MM
10-27-98	Due to temp sensor malfunction (replaced 11/15/98)		MM
10-29-98	which was temp/humidity recorder CT 495-AL 5" 026 965 used to record temp & humidity		MM
10-30-98	24	R	MM
11-3-98	24	R	MM
11-4-98	24	R	MM
11-20-98	24	R	MM

R = Routine; NR = Nonroutine (as defined by equipment SOP); NR entry may be made by service personnel; Q = Qualification

7569-98-0080-AC

DATE: 12/15/98  
SIGNATURE: *MM*