FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism:

Listeria monocytogenes (ATCC 19117)

PRODUCT IDENTITY

MDF 500 Parts A & B
Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30
and
Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02

DATA REQUIREMENTS

U.S. EPA 40 CFR Part 158
"Data Requirements for Registration"
Pesticide Assessment Guidelines - Subdivision G, 91-2 (i)

AUTHOR

Anne Stemper, B.S.
Study Director

STUDY COMPLETION DATE

March 9, 2007

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Modec, Inc.
4725 Oakland Street
Denver, CO 80239

PROJECT NUMBER

A04714

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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

Company: Modec, Inc.

Company Agent: James E. Tellman

CFO

Title

Signature

Date: 3/19/07

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Submitter: _____ Date: _____

Sponsor: _____ Date: 3/19/07
JAMES E. TELLMAN

Study Director: _____ Date: 3-9-07
Anne Stemper, B.S.

ATS LABS

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Use-Dilution Method

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	February 22, 2007	February 22, 2007	March 9, 2007
Final Report	March 8, 2007	March 8, 2007	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: _____ Date: 3/9/07

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ATS LABS

STUDY PERSONNEL

STUDY DIRECTOR: Anne Stemper, B.S.

Professional personnel involved:

David Rottjakob, M.T.	- Director, Microbiology Services
Scott R. Steinagel, B.S.	- Microbiology Laboratory Supervisor
Matthew Sathe, B.S.	- Research Assistant I
Peter Toll, B.S.	- Research Assistant I
Lisa Slusser, B.S.	- Research Assistant I
Katherine C. Sager, B.S.	- Research Assistant I
Jessica Rice, B.A.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A04714

Protocol Number: MOD02020907.UD

Sponsor: Modec, Inc.
4725 Oakland Street
Denver, CO 80239

Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: MDF 500 Parts A & B

Lot/Batchs: Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30
and
Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02

Test Substance Characterization
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: February 12, 2007
Study Initiation Date: February 19, 2007
Experimental Start Date: February 22, 2007
Experimental End Date: March 1, 2007
Study Completion Date: March 9, 2007

OBJECTIVE

The objective of this study was to determine the efficacy of the Sponsor's product following the AOAC Use-Dilution Method in compliance with the U.S. Environmental Protection Agency requirements set forth in the Pesticide Assessment Guidelines.

SUMMARY OF RESULTS

Test Substance:	MDF 500 Parts A & B, Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30 and Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02
Dilution:	Mix Equal Parts of Part A Lot 1 and Part B Lot 1 and Mix Equal Parts of Part A Lot 2 and Part B Lot 2
Test Organism:	*Listeria monocytogenes* (ATCC 19117)
Exposure Time:	Ten minutes
Exposure Temperature:	20 ± 1°C (20.5°C)
Organic Soil Load:	None required
Efficacy Result:	MDF 500 Parts A & B demonstrated efficacy of two lots against *Listeria monocytogenes*, and therefore, meets the requirements set forth by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
Listeria monocytogenes	19117	Brain Heart Infusion Broth

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media

Neutralizing Subculture Medium:	Primary and Secondary: Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.05% Catalase
Agar Plate Medium:	Tryptic Soy Agar with 5% Sheep Blood (BAP)

Carriers

Stainless steel penicylinders were pre-soaked overnight in 1.0 N NaOH, washed in water until rinse water was neutral to phenolphthalein, and autoclaved in 0.1% asparagine.

TEST METHOD

Preparation of Test Substance

A sample of MDF 500 Lot 1 was prepared by mixing 60.0 mL of Part A Lot 1 Batch AZB-30 with 60.0 mL of Part B Lot 1 Batch BZB-30. A sample of MDF 500 Lot 2 was prepared by mixing 60.0 mL of Part A Lot 2 Batch AXA-02 with 60.0 mL of Part B Lot 2 Batch BXB-02. Each lot of prepared test substance was homogenous as determined by visual observation.

Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20.5°C water bath and allowed to equilibrate for ≥10 minutes.

Preparation of Test Organism

From a stock slant, an initial tube of culture broth was inoculated. From this initial broth suspension a minimum of three daily transfers were performed on consecutive days prior to use in testing procedure. The appropriate growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) of the test organism.

A 48-54 hour broth culture incubated at 35-37°C was prepared. The test cultures were thoroughly mixed and allowed to stand for ≥10 minutes prior to use.

Contamination of Carriers

Sterile penicylinders were immersed for 15 minutes in a 48-54 hour old broth culture of the test organism, at a ratio of 1 carrier per 1 mL broth. The penicylinders were then dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes at a 40% relative humidity.

Exposure Conditions

For each prepared test substance, 10 contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the prepared test substance at the requested dilution and exposed for ten minutes at 20.5°C.

Test System Recovery

Following exposure, each exposed carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.05% Catalase. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 + 0.05% Catalase ≥30 minutes after subculture of the first carrier.

Incubation and Observation

The neutralized subculture tubes and plates were incubated for 48±4 hours at 35-37°C. Subcultures were stored at 2-8°C for two days prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of visible growth.

Representative subculture tubes demonstrating growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" was performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Carrier Sterility Control
A representative uninoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier was added to the subculture medium. The subculture medium containing the carrier was incubated and visually examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control
Ten percent of the secondary subculture tubes containing carriers showing no growth were inoculated with ≤100 CFU of the test organism and incubated. This control was performed with multiple replicates representing different dilutions of the test organism. A standardized spread plate procedure was run concurrently in order to enumerate the number of CFU actually added. The control result was reported using data from the most appropriate dilution.

The acceptance criterion for this study control is growth after inoculation with ≤100 CFU.

Carrier Population Control
Inoculated carriers were added at a ratio of 1 carrier to 10 mL neutralizing broth and vortex mixed. Appropriate serial ten-fold dilutions were prepared and aliquots were spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies were enumerated and the CFU/carrier calculated. The acceptance criterion for this study control is a minimum of 1.0×10^4 CFU/carrier.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The EPA efficacy performance requirements for label claims state that the disinfectant must kill the microorganism on 10 out of the 10 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

Carrier Population Control Calculation:

$$\text{CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralizer)}}{\text{(number of carriers tested) x (volume plated)}}$$

The carrier population was calculated and reported using data from the most appropriate dilution(s).

Statistical Analysis
None used.

STUDY RETENTION

Record Retention
All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. The original data includes, but is not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention
The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. Association of Official Analytical Chemists (AOAC), 1990. Use-Dilution Tests, p. 135-137. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

2. Association of Official Analytical Chemists (AOAC), 1990. Germicidal and Detergent Sanitizing Action of Disinfectants, p. 139 [Preparation of Synthetic Hard Water]. *In* Official Methods of Analysis of the AOAC, Fifteenth Edition.

3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.

4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.

5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A: Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including the culture purity, viability, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation, and carrier population were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

MDF 500 Parts A & B, Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30, mixed in equal parts of Part A Lot 1 and Part B Lot 1, demonstrated no growth of *Listeria monocytogenes* (ATCC 19117) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following a ten minute exposure period.

MDF 500 Parts A & B, Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02, mixed in equal parts of Part A Lot 2 and Part B Lot 2, demonstrated no growth of *Listeria monocytogenes* (ATCC 19117) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following a ten minute exposure period.

STUDY CONCLUSION

Under the conditions of this investigation, MDF 500 Parts A & B, Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30, mixed in equal parts of Part A Lot 1 and Part B Lot 1, demonstrated efficacy against *Listeria monocytogenes* as required by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

Under the conditions of this investigation, MDF 500 Parts A & B, Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02, mixed in equal parts of Part A Lot 2 and Part B Lot 2, demonstrated efficacy against *Listeria monocytogenes* as required by the U.S. EPA for disinfectant label claims following a ten minute exposure period.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results
	***Listeria monocytogenes* (ATCC 19117)**
Purity Control	Pure
Viability Control	Growth
Neutralizing Subculture Medium Sterility Control	No Growth
Carrier Sterility Control	No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
Listeria monocytogenes (ATCC 19117)	2-22-07	1.56×10^8 CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Average Inoculum (CFU/mL)	Number of Subculture Tubes	
				Tested	Positive
MDF500 Parts A & B, Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30	*Listeria monocytogenes* (ATCC 19117)	2-27-07	32	1	1
MDF500 Parts A & B, Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02				1	1

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution	Number of Carriers	
				Exposed	Showing Growth**
MDF 500 Parts A & B, Part A Lot 1 Batch AZB-30 + Part B Lot 1 Batch BZB-30	*Listeria monocytogenes* (ATCC 19117)	2-22-07	Equal Parts of Part A Lot 1 Batch AZB-30 and Part B Lot 1 Batch BZB-30	1°=10 2°=10	1°=0 2°=0
MDF 500 Parts A & B, Part A Lot 2 Batch AXA-02 + Part B Lot 2 Batch BXB-02			Equal Parts of Part A Lot 2 Batch AXA-02 and Part B Lot 2 Batch BXB-02	1°=10 2°=10	1°=0 2°=0

** Number of carriers showing growth of the test organism.
1° Primary Subculture
2° Secondary Subculture