

FINAL STUDY REPORT

PROTOCOL TITLE

AOAC Fungicidal Activity Method

Organism:

Trichophyton mentagrophytes

PROTOCOL NUMBER

SRC27073004.FACT

PRODUCT IDENTITY

ACCEL TB
Lot 2-3646-REG-US and Lot 3-3647-REG-US

DATA REQUIREMENTS

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

PROJECT NUMBER

A02357

AUTHOR

Jill Ruhme, B.S.
Study Director

STUDY COMPLETION DATE

September 29, 2004

PERFORMING LABORATORY

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

SPONSOR

Virox Technologies
2815 Bristol Circle, Unit 4
Oakville, Ontario L6H 6X5

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
102 1/2 South Chauncey Street
Columbia City, IN 46725-2306



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: Virox Technologies

Company Agent: Sally Hayes

Agent for Virox Technologies
Title

Sally Hayes
Signature

Date: 10/11/04

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: Sally Hayes
Sally Hayes, Agent for Virox Technologies

Date: 10/11/04

Sponsor: Ann M. Cozad
Ann M. Cozad, Agent for Virox Technologies

Date: 10/11/04

Study Director: Jill Ruhme
Jill Ruhme, B.S.

Date: 9-29-04

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Fungicidal Activity 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 date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	August 13, 2004	August 13, 2004	September 10, 2004
Draft Report	September 9, 2004	September 9, 2004	
Final Report	September 29, 2004	September 29, 2004	September 29, 2004

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

Quality Assurance Auditor: Rachelle L. Evenson Date: 09/29/04

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STUDY PERSONNEL

STUDY DIRECTOR: Jill Ruhme, B.S.

Professional personnel involved:

- | | |
|----------------------------|--------------------------------------|
| Douglas G. Anderson, Ph.D. | - President |
| Karen M. Ramm, B.A. | - Technical Director |
| David Rottjakob, M.T. | - Microbiology Program Manager |
| Scott R. Steinagel, B.S. | - Microbiology Laboratory Supervisor |
| Jill Ruhme, B.S. | - Research Scientist I |
| Matthew Sathe, B.S. | - Research Assistant I |
| Lisa Slusser, B.S. | - Research Assistant I |

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Fungicidal Activity Method
Project Number: A02357
Protocol Number: SRC27073004.FACT
Sponsor: Virox Technologies
2815 Bristol Circle, Unit 4
Oakville, Ontario L6H 6X5
Sponsor Representative: Scientific & Regulatory Consultants, Inc.
102 1/2 South Chauncey Street
Columbia City, IN 46725-2306
Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: ACCEL TB
Lot/Batch(s): Lot 2-3646-REG-US and Lot 3-3647-REG-US

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: March 11, 2004
Study Initiation Date: August 9, 2004
Experimental Start Date: August 13, 2004
Experimental End Date: August 23, 2004
Study Completion Date: September 29, 2004

OBJECTIVE

The objective of this study was to determine the efficacy of the Sponsor's product for fungicidal disinfection of inanimate objects.

SUMMARY OF RESULTS

Test Substance: ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US)

Dilution: Ready to use (RTU)

Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)

Exposure Time: 5, 10, 15 minutes

Exposure Temperature: 20±1°C

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: ACCEL TB demonstrated efficacy of two lots against *Trichophyton mentagrophytes* as required by the U.S. EPA for disinfectant label claims at the 5, 10 and 15 minute exposure times.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
<i>Trichophyton mentagrophytes</i>	9533	Potato Dextrose Agar

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

Recovery Media

Neutralizing Subculture Medium: Sabouraud Dextrose Broth with 1.0% Sodium Thiosulfate (primary)
 Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 (secondary)

Agar Plate Medium: Potato Dextrose Agar

Reagents

Organic Soil Load Description: 5% fetal bovine serum (FBS) added to conidial suspension

TEST METHOD

Preparation of Test Organism

A culture of *Trichophyton mentagrophytes* was prepared by inoculating agar plates at center and incubating at 25-30°C for 10-15 days. The mycelia was removed from all plates using a sterile swab. The mycelia was transferred to a glass bottle containing beads and a saline/Triton Solution (0.85% Saline + 0.05 % Triton X-100) and vortex mixed. The culture was filtered through sterile gauze to remove hyphal fragments. The conidial concentration was estimated by counting in a hemacytometer. The count was 1.6×10^8 conidia/mL.

Addition of Organic Soil Load

A 0.25 mL aliquot of FBS was added to 4.75 mL of conidial suspension to yield a 5% fetal bovine serum soil load.

Preparation of Test Substance

The test substance was ready to use. The test substance was homogenous as determined by visual observation.

Five (5) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a $20 \pm 1^\circ\text{C}$ water bath and allowed to equilibrate for ≥ 10 minutes.

Inoculation of Test Substance

A volume of 0.5 mL of the test organism suspension was added to the test substance. To inoculate the test substance, the tube was removed from the water bath and slanted slightly. The pipette was then inserted into the tube and the suspension was added without touching pipette into the fluid. The tube was agitated gently after adding the suspension and replaced into the water bath.

Subculture of Disinfectant

Exactly 5 minutes after transfer of organism suspension to each test substance, a 4 mm loop was inserted into slanted tube (60° angle) and sample was withdrawn without touching tube walls or lip. One (1) loopful was transferred to an appropriately labeled subculture tube containing 10 mL of broth media. Subcultures were repeated beginning at exactly 10 minutes and once again at exactly 15 minutes after transfer of culture to the test substance. Secondary subcultures were performed ≥ 30 minutes after initial subculture by transferring one loopful from each subculture tube into a similarly labeled secondary subculture tube to avoid fungistatic action.

Incubation and Observation

Subculture plates were incubated for 44-76 hours at 25-30°C.

Subculture tubes were incubated for 10 days at 25-30°C.

Following incubation, the subculture tubes were visually examined for growth.

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.

Organic Soil Sterility Control

The serum used for soil load was cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Viability Control

One loopful of the conidial suspension was transferred to a tube of subculture broth to demonstrate culture viability.

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.

Initial Suspension

This suspension was serially diluted and plated using standard microbiological techniques. Following incubation at 25-30°C for 44-76 hours, the organism plates were observed to enumerate the concentration of the test organism present at the time of testing. The initial suspension should contain $\geq 5 \times 10^6$ conidia/mL per AOAC method.

Neutralization Confirmation

The neutralization of the test substance was confirmed by transferring 0.01 mL of the test substance to primary subculture tubes containing 10 mL of subculture media. A secondary subtransfer from the initial subculture was performed ≥ 30 minutes after initial subculture as in test. The secondary transfer tubes were challenged with low levels of the organism (neutralization control), incubated as in test and observed for the presence of growth. Dilutions of the organism used for inoculation were plated on subculture agar to enumerate the number of organisms added to the subculture tubes.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

From all subculture tubes, growth (+) or no growth (0) was recorded. The test substance must kill conidial spores within 10 minutes to be an effective fungicide.

Control Acceptance Criteria

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

PROTOCOL CHANGES

Protocol Amendments:

1. Per Sponsor's request, this protocol is amended to correct the expiration date to March 8, 2005.
2. This protocol is amended to add the following viability control that was omitted from the protocol:
 One loopful of the conidial suspension will be transferred to a tube of subculture broth to demonstrate culture viability.

Protocol Deviations:

Although the protocol states that all subculture plates will be incubated for 10 days, the plates in this study were incubated for approximately 72 hours. The protocol should have stated "the subculture plates will be incubated for 44-76 hours." Because there was adequate growth of the test organism all study control plates, this deviation did not affect the validity of the test.

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. AOAC Official Methods of Analysis, Fungicidal Activity of Disinfectants, 955.17, 2000.
2. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-6, August 12, 1981.

RESULTS

For Control and Neutralization Results, see Tables 1-3.

All data measurements/controls including the culture purity, viability, organic soil load sterility, neutralizing subculture medium sterility, neutralization confirmation, and initial suspension were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated no growth of *Trichophyton mentagrophytes* in the primary and secondary subculture tubes at 5, 10 and 15 minutes in the presence of a 5% fetal bovine serum organic soil load at 20±1°C.

STUDY CONCLUSION

Under the conditions of this investigation, ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, was FUNGICIDAL when tested against *Trichophyton mentagrophytes* following 5, 10, and 15 minute exposure periods in the presence of a 5% fetal bovine serum organic soil load at 20±1°C.

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

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control		Results
		<i>Trichophyton mentagrophytes</i>
Culture Purity		Pure
Viability Control		Growth
Soil Sterility Control		No Growth
Neutralizing Broth Sterility Control	Primary	No Growth
	Secondary	No Growth

TABLE 2: NEUTRALIZATION CONFIRMATION RESULTS

Test Substance	Test Organism	Neutralization Confirmation			
		Date Performed	Inoculum CFU	Number Subculture Tubes Tested	Number Subculture Tubes Positive
ACCEL TB, Lot 2-3646-REG-US	<i>Trichophyton mentagrophytes</i>	8/13/04	83	1	1
ACCEL TB, Lot 3-3647-REG-US				1	1

CFU = Colony Forming Unit

The neutralization controls showed growth, eliminating fungistasis as a cause of lack of growth in the test system.

TABLE 3: INITIAL SUSPENSION POPULATION

Test Organism	Date Performed	Result
		Conidia/mL
<i>Trichophyton mentagrophytes</i>	8/13/04	8.3 x 10 ⁷

TABLE 4: EVALUATION OF GROWTH IN SUBCULTURES

Test Substance	Test Organism	Sample Dilution*	Exposure Time		
			5 Minutes	10 Minutes	15 Minutes
ACCEL TB, Lot 2-3646- REG-US	<i>Trichophyton mentagrophytes</i>	RTU	1°=0	1°=0	1°=0
			2°=0	2°=0	2°=0
1°=0			1°=0	1°=0	
2°=0			2°=0	2°=0	
ACCEL TB, Lot 3-3647- REG-US					

* RTU = Ready to use.
 0 = No growth in subculture tubes.
 1° = Primary subculture
 2° = Secondary subculture