



FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism:

Enterococcus faecalis Vancomycin Resistant (ATCC 51575)

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, 91-2 (i)

AUTHOR

Jill Ruhme, B.S.
Study Director

STUDY COMPLETION DATE

August 4, 2004

PERFORMING LABORATORY

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1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Virox Technologies
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Mississauga, Ontario L5N5M4

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
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PROJECT NUMBER

A02225

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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: 09/20/04

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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: 09/20/04

Sponsor: Rhonda Jones
Rhonda Jones, Agent for Virox Technologies

Date: 8-16-04

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

Date: 8-4-04

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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 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	June 22, 2004	June 22, 2004	July 28, 2004
Draft Report	July 26, 2004	July 27, 2004	
Final Report	August 3, 2004	August 3, 2004	August 4, 2004

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

Quality Assurance Auditor: Brenda Sjo Date: 8/4/04

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STUDY PERSONNEL

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- Microbiology Program Manager

- Microbiology Laboratory Supervisor

- Research Scientist I

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STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method
Project Number: A02225
Protocol Number: SRC27022404.UD.4
Sponsor: Virox Technologies
6705 Mill Creek Road Unit 4
Mississauga, Ontario L5N5M4
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: June 2, 2004
Experimental Start Date: June 22, 2004
Experimental End Date: June 28, 2004
Study Completion Date: August 4, 2004

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.

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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: *Enterococcus faecalis* Vancomycin Resistant (ATCC 51575)

Exposure Time: One minute

Exposure Temperature: 20±1°C

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Two lots of Accel TB demonstrated efficacy against *Enterococcus faecalis* Vancomycin Resistant as required by the U.S. EPA for disinfectant label claims.

STUDY MATERIALS

Test System/Growth Media

Test Organism	ATCC #	Growth Medium
<i>Enterococcus faecalis</i> Vancomycin Resistant	51575	Fluid Thioglycollate Medium

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

Recovery Media

Neutralizing Subculture Medium: Lethen Broth with 1.0% Sodium Thiosulfate (primary)
 Lethen Broth with 0.07% Lecithin + 0.5% Tween 80 (secondary)

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

Reagents

Organic Soil Load Description: 5% fetal bovine serum (FBS)

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.

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TEST METHOD

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. The test substance was homogenous as determined by visual observation.

Ten (10) mL of the test substance at its use dilution were aliquoted into sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing.

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 was 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. Antimicrobial susceptibility testing was performed utilizing a representative culture from the day of testing to verify the antimicrobial resistance pattern stated.

Addition of Organic Soil Load

A 1.3 mL aliquot of FBS was added to 24.7 mL of broth culture to yield a 5% fetal bovine serum soil load.

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 1.0 mL broth. The penicylinders were then dried on filter paper in a sterile petri dish at 35-37°C for 40 minutes at 48.7% relative humidity.

Exposure Conditions

For each test substance, 10 contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10 mL of the test substance at the requested dilution and exposed for one minute at 20±1°C.

Test System Recovery

Following exposure, each exposed carrier was then transferred by hook needle at identical staggered intervals to 10 mL of Lethen Broth with 1.0% Sodium Thiosulfate. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Lethen Broth with 0.07% Lecithin + 0.5% Tween 80 30-60 minutes following the first transfer.

Incubation and Observation

The neutralized subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth.

Representative neutralized subcultures showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

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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 observed for growth. The acceptance criterion for this study control is lack of growth.

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 observed. 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 examined for growth. The acceptance criterion for this study control is growth.

Neutralization Confirmation Control

The neutralization of the test substance was confirmed by exposing sterile carriers (representing not less than 10% of the total number of test carriers) to the test substance and transferring them to primary subculture tubes containing 10 mL of neutralizing subculture medium. Carriers were then transferred from primary subculture tubes into individual secondary subculture tubes 30-60 minutes following the primary transfer. The subculture tubes containing the exposed carriers were inoculated with ≤ 100 CFU of the test organism, incubated under test conditions and observed for the presence of growth. This control was performed with multiple replicates using 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 $\geq 1 \times 10^4$ CFU/carrier per EPA.

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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:

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

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

Statistical Analysis

None used.

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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, organic soil load sterility, neutralizing subculture medium sterility, carrier sterility, neutralization confirmation, and carrier population were within acceptance criteria.

For Test Results, see Table 4.

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ANALYSIS

Accel TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated no growth of *Enterococcus faecalis* Vancomycin Resistant (ATCC 51575) in any of the 10 primary subculture tubes and no growth in any of the 10 secondary subculture tubes following a one minute exposure period at $20\pm 1^{\circ}\text{C}$ in the presence of a 5% fetal bovine serum organic soil load.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum organic soil load, Accel TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated efficacy against *Enterococcus faecalis* Vancomycin Resistant after a one minute contact at $20\pm 1^{\circ}\text{C}$ as required by the U.S. EPA for disinfectant label claims.

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

The use of the ATS Labs name, logo or any other representation of ATS Labs without the written approval of ATS Labs is prohibited. In addition, ATS Labs may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express written permission of ATS Labs.

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results	
	<i>Enterococcus faecalis</i> Vancomycin Resistant (ATCC 51575)	
Purity Control	Pure	
Viability Control	Growth	
Organic Soil Sterility Control	No Growth	
Neutralizing Subculture	Primary	No Growth
Medium Sterility Control	Secondary	No Growth
Carrier Sterility Control	No Growth	

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism	Date Performed	Result
<i>Enterococcus faecalis</i> Vancomycin Resistant (ATCC 51575)	6/22/04	4.6 x 10 ⁶ CFU/carrier

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Date Performed	Inoculum (CFU/mL)	Number of Subculture Tubes Tested	Number of Subculture Tubes Positive
Accel TB, Lot 2-3646-REG-US	<i>Enterococcus faecalis</i> Vancomycin Resistant (ATCC 51575)	6/22/04	2	1	1
Accel TB, Lot 3-3647-REG-US				1	1

CFU = Colony Forming Unit

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Date Performed	Sample Dilution*	Number of Carriers	
				Exposed	Showing Growth**
Accel TB, Lot 2-3646-REG-US	<i>Enterococcus faecalis</i> Vancomycin Resistant (ATCC 51575)	6/22/04	RTU	1°=10	1°=0
Accel TB, Lot 3-3647-REG-US				2°=10	2°=0
				1°=10	1°=0
				2°=10	2°=0

* RTU = Ready to use.

** Number of carriers showing growth of the test organism.

1° Primary Subculture

2° Secondary Subculture

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ATTACHMENT I

Verification of Antibiotic Resistance

The following organism, Vancomycin Resistant *Enterococcus faecalis* (ATCC 51575), was purchased from the American Type Culture Collection (ATCC) by ATS Labs. ATS Labs verified that the organism was resistant by performing a Kirby Bauer Susceptibility assay under GLP conditions. The organism was subcultured onto a BAP plate and was incubated for approximately 24 hours at 35-37°C. Following incubation, a suspension of the test organism equal to a 0.5 McFarland Standard was made in 0.85% sterile saline. The suspension was streaked onto Mueller Hinton agar. A vancomycin disc was placed in the center of the inoculated Mueller Hinton plate. The plate was inverted and incubated for ≥ 24 hours at 35-37°C. Following incubation, the zone of inhibition was measured using a calibrated caliper. A control organism, *Staphylococcus aureus* (ATCC 25923), was run concurrently with the test organism to confirm the validity of the assay. The interpretation of the zone of inhibition is based on established NCCLS performance standards.

Organism (ATCC)	Zone of Inhibition (mm)	NCCLS* Resistant Range (mm)
Vancomycin Resistant <i>Enterococcus faecalis</i> – VRE (ATCC 51575)	0	≤ 14
Quality Control Organism	Zone of Inhibition (mm)	NCCLS* Acceptable Range (mm)
<i>Staphylococcus aureus</i> (ATCC 25923)	18	17-21

*NCCLS = National Committee for Clinical Laboratory Standards