

FINAL STUDY REPORT

PROTOCOL TITLE

Quantitative Tuberculocidal Suspension Method

PROTOCOL NUMBER

SRC27022404.QTB

PRODUCT IDENTITY

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

DATA REQUIREMENTS

US EPA 40 CFR Part 158
"Data Requirement for Registration"
Pesticide Assessment Guidelines - Subdivision G, Section 91-2 (g)

PROJECT NUMBER

A02040

AUTHOR

David Rottjakob, M.T.
Study Director

STUDY COMPLETION DATE

July 20, 2004

PERFORMING LABORATORY

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

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



STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

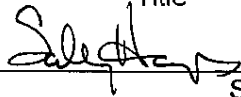
No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d) (1) (A), (B), or (C).

Company: Virox Technologies

Company Agent: Sally Hayes

Agent For Virox Technologies

Title


Signature

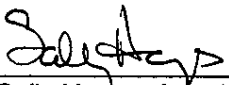
Date: 09/20/04

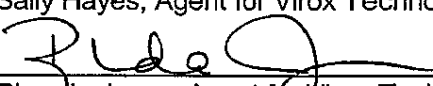



GOOD LABORATORY PRACTICE STATEMENT

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:  Date: 09/20/04
 Sally Hayes, Agent for Virox Technologies

Sponsor:  Date: 7-23-04
 Rhonda Jones, Agent for Virox Technologies

Study Director:  Date: 7/20/04
 David Rottjakob, M.T.

QUALITY ASSURANCE UNIT SUMMARY

Study: Quantitative Tuberculocidal Suspension 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 | March 30, 2004 | March 30, 2004 | May 20, 2004 |
| Draft Report | May 20, 2004 | May 20, 2004 | |
| Final Report | July 19, 2004 | July 19, 2004 | July 20, 2004 |

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

Quality Assurance Auditor: Rachelle L. Swann

Date: 07/20/04



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STUDY PERSONNEL

STUDY DIRECTOR: David Rottjakob, M.T.

Professional personnel involved:

- | | |
|----------------------------|--------------------------------------|
| Douglas G. Anderson, Ph.D. | - President |
| Karen M. Ramm, B.A. | - Technical Director |
| David Rottjakob, M.T. | - Microbiology Program Manager |
| Barbara Bailey, A.A. | - Microbiology Laboratory Supervisor |
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| Lisa Slusser, B.S. | - Research Assistant I |

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Quantitative Tuberculocidal Suspension Method

Project Number: A02040

Protocol Number: SRC27022404.QTB

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: March 15, 2004

Experimental Start Date: March 30, 2004

Experimental End Date: April 15, 2004

Study Completion Date: July 20, 2004

OBJECTIVE

The objective of this study was to determine the tuberculocidal effectiveness of a disinfectant following a modification of the EPA Guidelines for the Quantitative Tuberculocidal Procedure.

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: *Mycobacterium bovis* - BCG (OT 451C150)

Exposure Time: 5 minutes, 10 minutes, 15 minutes and 20 minutes

Exposure Temperature: 20±1°C

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: Two lots of ACCEL TB demonstrated efficacy against *Mycobacterium bovis* – BCG at 5, 10, 15 and 20 minutes as required by the U.S. EPA for disinfectant label claims.

STUDY MATERIALS

Test System/Growth Media

| Test Organism | OT # | Growth Medium | Incubation Parameters |
|----------------------------------|---------|-------------------------|-----------------------|
| <i>Mycobacterium bovis</i> - BCG | 451C150 | 7H9 Broth with Tween 80 | 35-37°C, aerobic |

The microorganism used in this study was obtained from Organon Teknika Corporation, Durham, NC.

Recovery Medium: Middlebrook 7H11 Agar Plates
 Neutralizer: Lethen Broth with 1.0% Sodium Thiosulfate

Reagents

Organic Soil Load Description: 5% fetal bovine serum (added to the organism suspension)

TEST METHOD

Preparation of Test Substance

The test substance was ready to use (RTU), as received from the Sponsor. The test substance was used undiluted and appeared homogeneous on day of test.

Preparation of Standardized Inoculum

One loopful of the control stock organism was transferred to Middlebrook 7H9 broth with Tween 80 and incubated at 35-37°C on a shaker until turbid (7-15 days). This suspension was then used to make a ≈10% inoculation into 7H9 broth with Tween 80 and incubated under identical conditions. On the day of testing this fresh suspension was homogenized using a tissue grinder and held in a 2 ± 2°C water bath.

Inoculation of Test

One tube containing 9.0 mL of test substance was equilibrated to testing temperature (19.0°C). One mL of the inoculum was added to the test substance and mixed. At time intervals specified by Sponsor (5, 10, 15 and 20 minutes), 1.0 mL aliquots of the test suspension were removed and added to 9.0 mL of neutralizer and mixed thoroughly. This represents the 10^{-1} dilution.

Treatment of Inoculated Test

The population of survivors was determined by making serial dilutions of the neutralized suspension in saline dilution blanks (10^{-1} through 10^{-7}). Ten mL of sterile saline was transferred into a $0.45\mu\text{m}$ filter membrane apparatus. Five (5) mL of the 10^{-1} dilution and 1 mL of the 10^{-3} , 10^{-5} , and 10^{-7} dilutions were added to the sterile saline in separate membrane apparatuses and gently mixed. Each mixture was filtered through the apparatus using a vacuum pump immediately following exposure. Each filter membrane was washed by adding 50 mL of sterile saline to the filter unit/pump apparatus.

Incubation and observation

Each filter membrane was removed aseptically from the filter unit and placed on the surface of a 7H11 agar plate. Plates were incubated at 35-37°C for 16 days in plastic bags. The survivors were determined and expressed as colony forming units per 10 mL of test mixture. The test mixture is defined as 9 mL test substance plus 1 mL organism suspension.

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

Neutralizer Sterility Control

A representative sample of neutralizer was incubated and observed. The acceptance criterion for this study control is lack of growth.

Subculture Medium Sterility Control

A representative agar plate was incubated and observed. The acceptance criterion for this study control is lack of growth.

Initial Suspension Population Control

The prepared test organism suspension was serially diluted and plated using standard microbiological techniques. Following incubation, the organism plates were observed to enumerate the concentration of the test organism inoculated into the test substance at the time of testing. There is no acceptance criterion for this study control.

Static Control

A static control was included to establish the effectiveness of the neutralizer. Nine-tenths (0.9) mL of test substance was added to 9.0 mL of neutralizer equilibrated to exposure temperature and mixed. One-tenth (0.1) mL of the inoculum was added to this solution and was treated identically to the test procedure. This procedure was repeated using sterile saline (saline blank control) in place of the aforementioned compounds and data was compared to the static control. The acceptance criterion for this study control requires the static control and corresponding population control results to be within 1.0 Log.

Toxicity Control

A toxicity control was included to demonstrate the neutralizer's lack of toxic effect on the test organisms at concentrations employed in this method. One tenth (0.1) mL of inoculum plus 0.9 mL diluent was added to nine mL of the neutralizer and mixed. The toxicity control was processed as the test substance. The acceptance criteria for this study control requires the toxicity neutralization control and corresponding population control results to be within 1.0 Log.

Neutralizer System Control

A neutralizer system control was included to demonstrate the effectiveness of the neutralizer in conjunction with the washing procedure in neutralizing the test substance. Nine-tenths (0.9) mL of the test substance was added to 9.0 mL neutralizer and 0.1 mL of sterile growth medium and mixed. This solution was filtered and washed as the 10^{-1} dilution. The filter was inoculated with approximately 100 CFU and evacuated and plated. The acceptance criteria for this study control requires the filtration neutralization control and corresponding population control results to be within 1.0 Log.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria

If required, survival curves are constructed to determine the tuberculocidal activity of the test agent. Data is plotted on semi-log axis as S/S_0 vs. time. S_0 is calculated by determining the viable count of the test organism culture for each replication, and S is the viable count at each time point for each replication. Each ratio for time points is averaged to generate data for a survival curve.

Survival curves are the average of at least four separate studies in order for upper 95% confidence limits to be determined for each point on the curve. The value for each upper 95% confidence limit is calculated by multiplying the standard deviation by 1.96. The minimum time that can be claimed for efficacy is determined by finding the point at which the average survival curve intersects the S/S_0 line on the graph which indicates the probability of one survivor.

This time also can be found by inspection of raw tabular data, locating the time at which an average of one organism or fewer is found. If inspection of the raw data is sufficient in determining the kill time a survival curve is not constructed. If the data show at least four \log_{10} kill of the starting population but the survivor curve does not intersect the one survivor S/S_0 line on graph, the minimum time that can be claimed is determined by extrapolating the upper 95% confidence limit curve to the one survivor line, using the last two points on the 95% confidence limit curve as a basis for the extrapolation. This extrapolation is considered valid only if the last data point demonstrates at least 99.99% ($4 \log_{10}$) kill.

The time established for TB efficacy claims should be in five minute increments. Where a zero survivor point or a 95% confidence limit intercept leads to a time other than that falling on a five minute increment, the claim is established by increasing the time up to the next higher five minute increment (e.g., if 22 minutes were a one survivor line intercept, the claim time would be 25 minutes. If an average zero point were found at 20 minutes, the claim time would be 20 minutes.)

Control Acceptance Criteria

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

PROTOCOL CHANGES

Protocol Amendment:

This protocol is amended for the following reason:

To rectify the incorrect test substance expiration date listed in the protocol, the protocol is amended to change the expiration date from March 9, 2005 to March 8, 2005.

Protocol Deviations:

No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation

Log Reduction = $-\text{Log}_{10}(\text{Number of survivors/Number of initial population}) = -\text{Log } S/S_0$

CFU/10 mL test mixture = $\frac{(\# \text{ of CFU}) (\text{Dilution Factor}) (\text{Volume of Neutralized Solution})}{(\text{Volume Filtered})}$

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. EPA Data call in documents, EPA Registration Division, June 13, 1986.
2. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A, 91-2 (g): Public Health Uses. In Pesticide Assessment Guidelines – Subdivision G (Product Performance).
3. 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.

RESULTS

For Control and Neutralization Results, see Tables 1-4

All data measurements/controls including the culture purity, static, organic soil sterility, neutralizer sterility, subculture medium sterility, toxicity and neutralizer system confirmation controls were within acceptance criteria.

For Test Results, see Table 5-6.

ANALYSIS

ACCEL TB, Lot 2-3646-REG-US, demonstrated a >6.80 log reduction at a 5 minute exposure, a >6.80 log reduction at 10 minutes, a >6.80 log reduction at 15 minutes, and a >6.80 log reduction at 20 minutes. Inspection of the data indicates ACCEL TB, Lot 2-3646-REG-US, demonstrated greater than a 4.0 log kill when tested against *Mycobacterium bovis* - BCG and therefore a survival curve was not constructed per EPA Guidance (Reference 1).

ACCEL TB, Lot 3-3647-REG-US, demonstrated a >6.80 log reduction at a 5 minute exposure, a >6.80 log reduction at 10 minutes, a >6.80 log reduction at 15 minutes, and a >6.80 log reduction at 20 minutes. Inspection of the data indicates ACCEL TB, Lot 3-3647-REG-US, demonstrated greater than a 4.0 log kill when tested against *Mycobacterium bovis* - BCG and therefore a survival curve was not constructed per EPA Guidance (Reference 1).

STUDY CONCLUSION

Under the conditions of this investigation, ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), is an effective tuberculocide at 5, 10, 15 and 20 minutes as required by the U.S. EPA for disinfectant label claims.

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TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

| Type of Control | Results |
|--------------------------------|----------------------------------|
| | <i>Mycobacterium bovis</i> - BCG |
| Purity Control | Pure |
| Organic Soil Sterility Control | No Growth |
| Neutralizer Sterility Control | No Growth |
| Subculture Medium Sterility | No Growth |

TABLE 2: INITIAL SUSPENSION CONTROL RESULTS

| Test Organism | Date Performed | Average CFU/mL |
|----------------------------------|----------------|------------------------|
| <i>Mycobacterium bovis</i> - BCG | 3/30/04 | 1.26 x 10 ⁸ |

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZER CONTROL

| Test Substance | Test Organism | Saline Control (CFU/mL) | Static Control (CFU/mL) | Log Difference Pass/Fail | Toxicity Control (CFU/mL) | Log Difference Pass/Fail |
|-----------------------------|----------------------------------|-------------------------|-------------------------|--------------------------|---------------------------|--------------------------|
| ACCEL TB, Lot 2-3646-REG-US | <i>Mycobacterium bovis</i> - BCG | 7.1×10^6 | 2.06×10^7 | -0.46 Pass | 1.74×10^7 | -0.39 Pass |
| ACCEL TB, Lot 3-3647-REG-US | | | 1.70×10^7 | -0.38 Pass | | |

TABLE 4: NEUTRALIZER SYSTEM CONTROL

| Test Substance | Test Organism | Dilution | Sample Results (CFU/plate) | Numbers Control @ 10^{-5} (CFU/plate) | Log Difference Pass/Fail |
|-----------------------------|----------------------------------|-----------|----------------------------|---|--------------------------|
| ACCEL TB, Lot 2-3646-REG-US | <i>Mycobacterium bovis</i> - BCG | 10^{-1} | 98 | 116 | 0.07 Pass |
| | | 10^{-3} | 112 | | 0.01 Pass |
| | | 10^{-5} | 108 | | 0.03 Pass |
| | | 10^{-7} | 110 | | 0.02 Pass |
| ACCEL TB, Lot 3-3647-REG-US | | 10^{-1} | 116 | | 0 Pass |
| | | 10^{-3} | 96 | | 0.08 Pass |
| | | 10^{-5} | 82 | | 0.15 Pass |
| | | 10^{-7} | 76 | | 0.72 Pass |

CFU = Colony Forming Unit

TABLE 5: TEST RESULTS

| Test Substance | Exposure Period (minutes) | Run # | (CFU Recovered/Filter) | | | | Number of Survivors (CFU/10 mL Test Mixture)* |
|-----------------------------|---------------------------|-------|------------------------|------------------|------------------|------------------|---|
| | | | 10 ⁻¹ | 10 ⁻³ | 10 ⁻⁵ | 10 ⁻⁷ | |
| ACCEL TB, Lot 2-3646-REG-US | 5 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 10 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 15 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 20 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| ACCEL TB, Lot 3-3647-REG-US | 5 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 10 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 15 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |
| | 20 minutes | 1 | 0 | 0 | 0 | 0 | <20 |
| | | 2 | 0 | 0 | 0 | 0 | <20 |
| | | 3 | 0 | 0 | 0 | 0 | <20 |
| | | 4 | 0 | 0 | 0 | 0 | <20 |

* Method detection limit is < 20 CFU/10mL

TABLE 6: CALCULATED RESULTS

| Test Substance | Exposure Period (minutes) | Average Number of Survivors (S)* CFU/10mL*** | S/S ₀ ** | Log Reduction (-Log S/S ₀) |
|------------------------------------|---------------------------|---|------------------------|---|
| ACCEL TB, Lot 2-3646- REG-US | 5 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 10 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 15 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 20 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| ACCEL TB, Lot 3-3647- REG-US | 5 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 10 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 15 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |
| | 20 minutes | <20 | $>1.59 \times 10^{-7}$ | >6.80 |

*S = Population at each time point.

**S₀ = Initial suspension population.

*** Method detection limit is < 20 CFU/10mL