



## FINAL STUDY REPORT

### PROTOCOL TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Virus: Rotavirus**

### DATA REQUIREMENT

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

### PRODUCT IDENTITY

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

### PROTOCOL NUMBER

SRC27022304.ROT

### PROJECT NUMBER

A02071

### AUTHOR

Karen M. Ramm, B.A.  
Study Director

### STUDY COMPLETION DATE

July 1, 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

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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: 09/27/04

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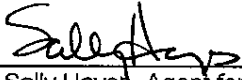
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### GOOD LABORATORY PRACTICE STATEMENT

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.

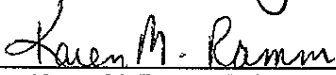
The procedures not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compound(s).

Submitter:   
Sally Hayes, Agent for Virox Technologies

Date: 09/27/04

Sponsor:   
Rhonda Jones, Agent for Virox Technologies

Date: 7-6-04

Study Director:   
Karen M. Ramm, B.A.

Date: 7-1-04

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### QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed under Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures and a standard protocol. 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	April 12, 2004	April 12, 2004	April 30, 2004
Draft Report	April 29, 2004	April 29, 2004	
Final Report	June 30, 2004	June 30, 2004	July 1, 2004

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

Quality Assurance Auditor: Rachelle L. Egan

Date: 07/01/04

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**STUDY PERSONNEL**

STUDY DIRECTOR: Karen M. Ramm, B.A.

Professional Personnel Involved:

Douglas G. Anderson, Ph.D.	- President
Karen M. Ramm, B.A.	- Technical Director
Mary J. Miller, M.T.	- Research Scientist II
Katherine A. Paulson, M.L.T.	- Research Assistant II

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

**Project Number:** A02071

**Protocol Number:** SRC27022304.ROT

**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

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

### TEST SUBSTANCE IDENTITY

**Test Substance:** 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, solubility, storage, etc., is the responsibility of the Sponsor.

### STUDY DATES

**Date Sample Received:** March 11, 2004  
**Study Initiation Date:** April 1, 2004  
**Experimental Start Date:** April 7, 2004  
**Experimental End Date:** April 14, 2004  
**Study Completion Date:** July 1, 2004

### OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against Rotavirus according to test criteria and methods approved by the United States Environmental Protection Agency (U.S. EPA) for registration of a product as a virucide.

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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)  
Virus: Rotavirus, Strain WA  
Exposure Time: One minute  
Exposure Temperature: 20±1°C  
Organic Soil Load: 5% fetal bovine serum  
Efficacy Result: Two lots of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Rotavirus under these test conditions as required by the U.S. EPA for claims of virucidal activity.

## TEST SYSTEM

- Virus  
The WA strain of Rotavirus used for this study was obtained from the University of Ottawa, Ontario, Canada. The stock virus was prepared by collecting the supernatant culture fluid from infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤ -70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot XR-110) was removed, thawed and refrigerated until use in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Rotavirus on MA-104 cells.
- Test Cell Cultures  
MA-104 (Rhesus monkey kidney) cells were obtained from ViroMed Laboratories, Inc., Cell Culture Division. Cultures were maintained and used as monolayers in disposable tissue culture labware. On the day of testing, cells were observed as having proper cell integrity and therefore, were acceptable for use in this study.
- Test Medium  
The test medium used for this assay was serum-free Eagles minimal essential medium (E-MEM) supplemented with 0.5µg/mL trypsin, 2.0 mM L-glutamine and the following: 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/ml amphotericin B (Fungizone). Cultures are maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>.

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The following table lists the test and control groups, the dilutions assayed, and the number of cultures used. See text for a more detailed explanation.

NUMBER OF DILUTIONS AND CULTURES FOR VIRUCIDAL EFFICACY STUDY			
Test or Control Group	Dilutions Assayed (log <sub>10</sub> )	Cultures per dilution	Total Cultures
Cell Control	N/A	4	4/group
Dried Virus Control (Group A)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample lot #1 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Sample lot #2 + virus (Group B)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #1 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Cytotoxicity of lot #2 (Group C)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #1 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28
Non-Virucidal level - lot #2 (Group D)	-1,-2,-3,-4,-5,-6,-7	4	28

## METHODS

- Preparation of Test Substance  
 Two lots of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) were used, undiluted, as received from the Sponsor. The test substance was in solution as determined by visual observation.
- Preparation of Virus Films  
 Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15mm sterile glass petri dishes. The virus films were dried at 20.1°C in a relative humidity of 42% until visibly dry (20 minutes).
- Sephadex Gel Filtration  
 To reduce the cytotoxic level of the virus-disinfectant mixture prior to assay of virus and/or to reduce the virucidal level of the disinfectant, virus was separated from disinfectant by filtration through Sephadex gel. Columns of Sephadex LH-20-100 were equilibrated with phosphate buffered saline containing 1% albumin, centrifuged for three minutes to clear the void volume, loaded with 2.0 mL of virus-disinfectant mixture and immediately passed through the column utilizing the syringe plunger.
- Treatment of Virus Films with Test Substance (GROUP B, TABLE 1)  
 For each lot of disinfectant, separate dried virus films were exposed to 2.0 mL of the use dilution for one minute at 20.0°C. Following the exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-disinfectant mixture was immediately passed through a Sephadex column utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10<sup>-1</sup> dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

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5. Treatment of Virus Control Films (GROUP A, TABLE 1)  
 A virus film was prepared as previously described (paragraph 2). The control film was exposed to 2.0 mL of test medium for the same amount of time as the test film was exposed to the disinfectant. The virus was then scraped and passed through a Sephadex column in the same manner as the test virus and the filtrate ( $10^{-1}$  dilution) was then titered by 10-fold serial dilution and assayed for infectivity.
6. Cytotoxicity Assay (GROUP C, TABLE 2)  
 A 2.0 mL aliquot of the use dilution of each lot of the disinfectant was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into MA-104 cell cultures. Cytotoxicity of the MA-104 cell cultures was scored at the same time as the virus-disinfectant and virus control cultures.
7. Assay of Non-Virucidal Level of Test Substance (GROUP D, TABLE 3)  
 Each dilution of the Sephadex-filtered disinfectant (disinfectant control for cytotoxicity assay) was mixed with an aliquot of low titer stock virus, and the resulting mixtures of dilutions were assayed for infectivity in order to determine the dilution(s) of disinfectant at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining the reduction in infectivity by the test substance.
8. Infectivity Assays  
 The MA-104 cell line, which exhibits CPE in the presence of Rotavirus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The inoculum was allowed to adsorb for 60 minutes at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>. Following the adsorption period, a 1.0 mL aliquot of test medium was added to the cell cultures and they were incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.
9. Statistical Methods: N/A

### PROTOCOL CHANGES

#### **Protocol Amendments:**

No protocol amendments were required for this study.

#### **Protocol Deviations:**

No protocol deviations occurred during this study.

### DATA ANALYSIS

#### **Calculation of Titers**

Viral and cytotoxicity titers are expressed as  $-\log_{10}$  of the 50 percent titration endpoint for infectivity (TCID<sub>50</sub>) or cytotoxicity (TCD<sub>50</sub>), respectively, as calculated by the method of Spearman Karber.

$$-\text{Log of 1st dilution inoculated} - \left[ \left( \left( \frac{\text{Sum of \% mortality at each dilution}}{100} \right) - 0.5 \right) \times (\text{logarithm of dilution}) \right]$$

#### **Calculation of Log Reduction**

Dried Virus Control TCID<sub>50</sub> – Test Substance TCID<sub>50</sub> = Log Reduction

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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. These original data include, but are 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. Annual Book of ASTM Standards 2000, Section 11 Water and Environmental Technology Volume 11.05 Biological Effects and Environmental Fate: Biotechnology; Pesticides, E1053-97.
2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G: Product Performance, 91-2(f), November 1982.
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-7, November 12, 1981.
4. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Schmidt, N.J. and Emmons, R.W. editors. Sixth edition, 1989. p. 18-20.
5. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.

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### **STUDY RESULTS**

Results of tests with two lots of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) exposed to Rotavirus for one minute in the presence of a 5% fetal bovine serum soil load at 20.0°C are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the dried virus control was 5.5 log<sub>10</sub>. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ( $\leq 0.5$  log<sub>10</sub>). Test substance cytotoxicity was not observed in either lot at any dilution tested ( $\leq 0.5$  log<sub>10</sub>). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at  $\leq 0.5$  log<sub>10</sub> for both lots. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was  $\geq 5.0$  log<sub>10</sub> for both lots.

### **STUDY CONCLUSION**

Under the conditions of this investigation, in the presence of a 5% fetal bovine serum soil load, ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US), ready to use, demonstrated complete inactivation of Rotavirus following a one minute exposure time at 20.0°C as required by the U.S. EPA for virucidal 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.

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**TABLE 1: Effects of ACCEL TB (Lot 2-3646-REG-US and Lot 3-3647-REG-US) Following a One Minute Exposure to Rotavirus Dried on an Inanimate Surface**

Dilution	Dried Virus Control (GROUP A)	Rotavirus + Lot 2-3646-REG-US (GROUP B)	Rotavirus + Lot 3-3647-REG-US (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	++++	0 0 0 0	0 0 0 0
10 <sup>-2</sup>	++++	0 0 0 0	0 0 0 0
10 <sup>-3</sup>	++++	0 0 0 0	0 0 0 0
10 <sup>-4</sup>	++++	0 0 0 0	0 0 0 0
10 <sup>-5</sup>	0+++	0 0 0 0	0 0 0 0
10 <sup>-6</sup>	0+00	0 0 0 0	0 0 0 0
10 <sup>-7</sup>	0 0 0 0	0 0 0 0	0 0 0 0
TCID <sub>50</sub> /0.1 mL	10 <sup>5.5</sup>	≤10 <sup>0.5</sup>	≤10 <sup>0.5</sup>

**TABLE 2: Cytotoxicity of ACCEL TB on MA-104 Cell Cultures**

Dilution	Cytotoxicity Control Lot 2-3646-REG-US (GROUP C)	Cytotoxicity Control Lot 3-3647-REG-US (GROUP C)
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	0 0 0 0	0 0 0 0
10 <sup>-2</sup>	0 0 0 0	0 0 0 0
10 <sup>-3</sup>	0 0 0 0	0 0 0 0
10 <sup>-4</sup>	0 0 0 0	0 0 0 0
10 <sup>-5</sup>	0 0 0 0	0 0 0 0
10 <sup>-6</sup>	0 0 0 0	0 0 0 0
10 <sup>-7</sup>	0 0 0 0	0 0 0 0
TCD <sub>50</sub> /0.1 mL	≤10 <sup>0.5</sup>	≤10 <sup>0.5</sup>

(+) = Positive for the presence of test virus

(0) = No test virus recovered and/or no cytotoxicity present

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**TABLE 3: Non-Virucidal Level of Test Substance (Neutralization Control)**

Dilution	Test Virus + Cytotoxicity Control Lot 2-3646-REG-US (GROUP D)	Test Virus + Cytotoxicity Control Lot 3-3647-REG-US (GROUP D)
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	++++	++++
10 <sup>-2</sup>	++++	++++
10 <sup>-3</sup>	++++	++++
10 <sup>-4</sup>	++++	++++
10 <sup>-5</sup>	++++	++++
10 <sup>-6</sup>	++++	++++
10 <sup>-7</sup>	++++	++++

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)  
 (0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at TCID<sub>50</sub> of  $\leq 0.5 \log_{10}$  for both lots.