FINAL STUDY REPORT

PROTOCOL TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Human Immunodeficiency Virus type 1

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.HIV

PROJECT NUMBER

A02066

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

June 3, 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

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

Sponsor: Rhonda Jones Date: 6-7-04
Rhonda Jones, Agent for Virox Technologies

Study Director: Mary J. Miller Date: 6-3-04
Mary J. Miller, M.T.

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 nonclinical 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 15, 2004	April 15, 2004	April 27, 2004
Draft Report	April 23, 2004	April 26, 2004	
Final Report	June 2, 2004	June 2, 2004	June 3, 2004

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

Quality Assurance Auditor: Rachelle L. Evenson Date: 06/03/04

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STUDY PERSONNEL

STUDY DIRECTOR: Mary J. Miller, M.T.

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: A02066

Protocol Number: SRC27022304.HIV

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 Name: ACCEL TB

Batch/Lot(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 15, 2004
Study Completion Date: June 3, 2004

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against Human Immunodeficiency Virus type 1 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
Batch/Lots:	Lot 2-3646-REG-US and Lot 3-3647-REG-US
Dilution:	Ready to use (RTU)
Virus:	Human Immunodeficiency Virus type 1, Strain HTLV-III$_B$
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 Human Immunodeficiency Virus type 1 under these test conditions as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

1. Virus
The HTLV-III$_B$ strain of Human Immunodeficiency Virus type 1 (HIV-1) used for this study was originally obtained from Advanced Biotechnologies, Inc., Columbia, Maryland. 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 2200 RPM for ten minutes at approximately 4°C. The supernatant was removed and the high titer stock virus was aliquoted and stored at ≤ -70°C until the day of use. On the day of use an aliquot of stock virus (ATS Labs Lot HIV-4) was removed, thawed and refrigerated until use in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of HIV on MT-2 cells.

2. Test Cell Cultures
MT-2 cells (human CD4+ lymphocytes) were originally obtained from the National Cancer Institute, Frederick, MD. Cultures were grown and propagated in-house and used in suspension 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.

3. Test Medium
The test medium used in this study was RPMI 1640 supplemented with 15% (v/v) heat-inactivated fetal bovine serum (FBS). The medium was also supplemented with 2.0 mM L-glutamine and 50 µg/mL gentamicin.

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_{10})**	**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

1. 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.

2. 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 room temperature (24.0°C) in a relative humidity of 16.8% until visibly dry (20 minutes) and then incubated at 36-38°C for an additional 30 minutes to increase the level of dryness.

3. Sephadex Gel Filtration
   To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus and/or to reduce the virucidal level of the test substance, virus was separated from the test substance 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-test substance mixture and immediately passed through the column utilizing the syringe plunger.

4. Treatment of Virus Films with Test Substance (GROUP B, TABLE 1)
   For each lot of test substance separate dried virus films were exposed to 2.0 mL of the use dilution for one minute at 20.5°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^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

5. Treatment of Virus Control Film (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 test substance. 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 test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into MT-2 cell cultures. Cytotoxicity of the MT-2 cell cultures was scored at the same time as the virus-test substance and virus control cultures.

7. Assay of Non-Virucidal Level of Test Substance (GROUP D, TABLE 3)
Each dilution of the Sephadex-filtered test substance (test substance 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 test substance 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 MT-2 cell line, which exhibits CPE in the presence of HIV-1, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions prepared from test and control groups. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. Cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 in sterile disposable cell culture labware. The cultures were scored periodically for eight 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 ($TCID_{50}$) or cytotoxicity (TCD_{50}), 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 $TCID_{50}$ – Test Substance $TCID_{50}$ = Log Reduction

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 the 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.
6. Techniques in HIV Research, A. Aldovini and B. Walker, 1990.

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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), ready to use, exposed to HIV-1 virus in the presence of a 5% fetal bovine serum soil load at 20.5°C for one minute 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 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ($\leq 1.5 \log_{10}$). Test substance cytotoxicity was observed in both lots at 1.5 $\log_{10}$. The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq 1.5 \log_{10}$ for both lots. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was $\geq 4.0 \log_{10}$ 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 Human Immunodeficiency Virus type 1 following a one minute exposure time at 20.5°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 HIV-1 Dried on an Inanimate Surface

Dilution	Dried Virus Control (GROUP A)	HIV-1 + Lot 2-3646-REG-US (GROUP B)	HIV-1 + Lot 3-3647-REG-US (GROUP B)
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	T T T T	T T T T
10^{-2}	+ + + +	0 0 0 0	0 0 0 0
10^{-3}	+ + + +	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	0 0 0 0	0 0 0 0
10^{-5}	+ 0 + +	0 0 0 0	0 0 0 0
10^{-6}	+ 0 0 0	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/0.2 mL	$10^{5.5}$	$\leq 10^{1.5}$	$\leq 10^{1.5}$

TABLE 2: Cytotoxicity of ACCEL TB on MT-2 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^{-1}	T T T T	T T T T
10^{-2}	0 0 0 0	0 0 0 0
10^{-3}	0 0 0 0	0 0 0 0
10^{-4}	0 0 0 0	0 0 0 0
10^{-5}	0 0 0 0	0 0 0 0
10^{-6}	0 0 0 0	0 0 0 0
10^{-7}	0 0 0 0	0 0 0 0
TCD_{50}/0.2 mL	$10^{1.5}$	$10^{1.5}$

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(T) = Cytotoxicity present

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^{-1}	T T T T	T T T T
10^{-2}	+ + + +	+ + + +
10^{-3}	+ + + +	+ + + +
10^{-4}	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +
10^{-7}	+ + + +	+ + + +

(+) = 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
(T) = Cytotoxicity present

Results of the non-virucidal level control indicate that the test substance was neutralized at $TCID_{50}$ of $\leq 1.5 \log_{10}$ for both lots.

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