



FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces
Duck Hepatitis B Confirmatory Assay

PRODUCT IDENTITY

ACCEL TB
Lot 2-3646-REG-US

PROTOCOL NUMBER

SRC27022304.DHBV.2

DATA REQUIREMENT

Environmental Protection Agency Federal Register: August 25, 2000
(Volume 65, Number 166)

AUTHOR

Mary J. Miller, M.T.
Study Director

STUDY COMPLETION DATE

September 30, 2004

PERFORMING LABORATORY

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

SPONSOR

Virox Technologies
2815 Bristol Circle, Unit 4
Oakville, Ontario L6H6X5

SPONSOR REPRESENTATIVE

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

PROJECT NUMBER

A02070

Page 1 of 16

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 2 of 16



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: 05/02/05

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 3 of 16



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 test substance(s).

Submitter: Sally Hayes
Sally Hayes, Agent for Virox Technologies

Date: 05/02/05

Sponsor: Rhonda Jones
Rhonda Jones, Agent for Virox Technologies

Date: 10-11-04

Study Director: Mary J. Miller
Mary J. Miller, M.T.

Date: 9-30-04

Project No. A02070
 Protocol Number: SRC27022304.DHBV.2

Virox Technologies
 Page 4 of 16



QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces
 Duck Hepatitis B Confirmatory Assay

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 dates listed below. This study was 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 8, 2004
Draft Report	September 7, 2004	September 7, 2004	
Final Report	September 29, 2004	September 29, 2004	September 30, 2004

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

Quality Assurance Auditor: Rachelle L. Erman Date: 09/30/04

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 5 of 16



TABLE OF CONTENTS

Title Page1

Statement of No Data Confidentiality Claims2

Good Laboratory Practice Statement.....3

Quality Assurance Unit Summary4

Table of Contents5

Study Personnel6

General Study Information.....7

Test Substance Identity7

Study Dates.....7

Objective7

Summary of Results8

Test System8

Test Method9

Study Acceptance Criteria11

Protocol Changes.....12

Data Analysis.....12

Study Retention.....13

References13

Study Results14

Study Conclusion.....14

Table 1: Virus Controls and Test Substance Assay Results15

Table 2: Test Substance Cytotoxicity and Neutralization Control Results15

Table 3: BTC 835 Assay Results.....16

Table 4: BTC 835 Cytotoxicity and Neutralization Control Results.....16

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 6 of 16



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
Dale L. Johnson, B.S.	- Research Assistant I

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 7 of 16



STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Duck Hepatitis B Confirmatory Assay

Project Number: A02070

Protocol Number: SRC27022304.DHBV.2

Sponsor: Virox Technologies
2815 Bristol Circle, Unit 4
Oakville, Ontario L6H6X5

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

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. The characterization of the BTC 835 validation control substance is the responsibility of the manufacturer. A certified copy of the Certificate of Analysis supplied by the manufacturer is maintained in the study file.

STUDY DATES

Date Sample Received: March 11, 2004
Study Initiation Date: April 1, 2004
Experimental Start Date: August 13, 2004
Experimental End Date: August 23, 2004
Study Completion Date: September 30, 2004

OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a disinfectant against duck Hepatitis B virus according to test criteria and methods approved by the U.S. Environmental Protection Agency (EPA) for registration of a product as virucide. This study followed the guidelines set forth in the Environmental Protection Agency Federal Register: August 25, 2000 (Volume 65, Number 166).

Human Hepatitis B virus (HBV), a member of the Hepadnaviridae Family of enveloped DNA-containing viruses, presents a serious public safety concern. However, at present, there is no reliable *in vitro* infectivity assay for these viruses and the most reliable *in vivo* system uses primates (chimpanzees). Duck Hepatitis B virus, also a member of Hepadnaviridae Family, serves as a valuable model virus for Human Hepatitis B virus, since these viruses share many similar characteristics.

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 8 of 16



SUMMARY OF RESULTS

Test Substance: ACCEL TB, Lot 2-3646-REG-US
Dilution: Ready to use (RTU)
Virus: Duck Hepatitis B virus
Exposure Time: One minute
Exposure Temperature: 20±1°C
Organic Soil Load: 100% Duck serum
Efficacy Result: One lot of ACCEL TB (Lot 2-3646-REG-US) met the test criteria specified in the study protocol. Under these test conditions, the results indicate **complete inactivation** of duck Hepatitis B virus as required by the U.S. EPA for claims of virucidal activity.

TEST SYSTEM

1. Virus

The DHBV16 strain of the duck Hepatitis B virus (DHBV) used in this assay was obtained from Hepadnavirus Testing, Palo Alto, CA and consists of duck Hepatitis B virus serum obtained from congenitally infected ducklings. The virus was stored at ≤ -70°C until the day of use. On the day of use, three aliquots of stock virus (Lot D2-H) were removed, thawed, combined and refrigerated until use in the assay. The stock virus culture contained 100% duck serum as the organic soil load. The stock virus tested demonstrated fluorescence typical of DHBV on primary duck hepatocytes.

2. Indicator Cell Cultures

Cultures of primary duck hepatocytes were obtained from the AAALAC accredited Minneapolis Veterinary Medical Unit Services located at the Department of Veterans Affairs Medical Center, Minneapolis, MN. The cultures were prepared utilizing ducklings received from Abendroth Hatchery, Waterloo, WI. Upon receipt, the ducklings were screened for the presence of the test virus via a polymerase chain reaction (PCR) specific for DHBV at ViroMed Laboratories, Inc. Only ducklings verified to be free of the test virus and less than seven days old were utilized in this assay. An *in situ* perfusion of the duck liver utilizing Williams' E medium containing collagenase was performed at the AAALAC accredited animal facility of Minneapolis Veterinary Medical Unit Services by properly trained personnel. The hepatocytes were then seeded into sterile six well disposable tissue culture labware by personnel from ATS Labs. The cultures were maintained and used at the appropriate density and incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂.

All cell culture documentation will be retained for the cell cultures used in this assay with respect to source, perfusion process, seeding densities, and the general condition of the cells.

3. Test Medium

Test medium used in this study was Leibovitz L-15 medium supplemented with 0.1% glucose, 10µM dexamethasone, 10µg/mL insulin, 20 mM HEPES, 10 µg/mL gentamicin, and 100 units/mL penicillin.

Project No. A02070
 Protocol Number: SRC27022304.DHBV.2

Virox Technologies
 Page 9 of 16



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

SAMPLES TESTED FOR THE PRESENCE OF VIRUS			
Test or Control Group	Dilutions Assayed Per Carrier (log₁₀)	Cultures per Dilution	Total Cultures Inoculated
Negative Controls	N/A	4	2-4/group
Input Virus Control	-4,-5,-6,-7	4	16
Dried Virus Control (performed in duplicate)	-4,-5,-6,-7	4	32
Test Sample - (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control – Test substance	-2,-3	2	4
Neutralization Control - Test substance	-2,-3	2	4
175 ppm BTC 835 Validation Control (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control - 175 ppm BTC 835	-2,-3	2	4
Neutralization Control - 175 ppm BTC 835	-2,-3	2	4
350 ppm BTC 835 Validation Control (performed in duplicate)	-2,-3,-4	4	24
Cytotoxicity Control - 350 ppm BTC 835	-2,-3	2	4
Neutralization Control - 350 ppm BTC 835	-2,-3	2	4

TEST METHOD

1. Preparation of Test Substance

One lot of ACCEL TB (Lot 2-3646-REG-US) was used, undiluted, as received from the Sponsor. The test substance was in solution as determined by visual observation.

2. Preparation of the BTC Validation Control

BTC 835 (EPA Registration # 1839-32) received from Stepan Company, Northfield, IL was utilized as the validation control substance. The manufacture date is August 28, 2003. The expiration date is August 28, 2005. A 175 parts per million (ppm) and a 350 ppm concentration of the control substances were made in sterile deionized water.

According to the certificate of analysis supplied with the control substance, the stock BTC 835 is a 50.21% concentration (502,100 ppm). From this stock solution, a 1:100 dilution of the stock BTC 835 was made by adding 1.0 mL of BTC 835 to a 100 mL volumetric flask and adding sterile deionized water to the 100 mL mark, which equals a 5021 ppm concentration. The 350 ppm concentration of BTC 835 used in the study was made by adding 1.0 mL of the 5021 ppm concentration of BTC 835 to 13.35 mL sterile deionized water. The 175 ppm concentration was prepared by adding 4.0 mL of the 350 ppm concentration of BTC 835 to 4.0 mL sterile deionized water. Both concentrations of the control were in solution as determined by visual observation and used on the day of preparation.

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 10 of 16



3. Preparation of Virus Films

Films of virus were prepared at staggered intervals by spreading 0.2 mL of virus inoculum uniformly over the bottoms of eight separate 100 X 15mm sterile glass petri dishes. The virus films were air-dried at 20.1°C in a relative humidity of 43% for 30 minutes. The virus films were dry as determined by visual observation.

4. 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.

5. Input Virus Control

On the day of test, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus.

6. Treatment of Virus Films with Test Substance

Two separate dried virus films were exposed to 2.0 mL of the use dilution of the test substance for the Sponsor specified exposure time of one minute at 20.1°C. Following the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and the virus-disinfectant mixtures were immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity.

7. Treatment of Virus Control Films

Two virus films were prepared as previously described. The control films were individually exposed to 2.0 mL of test medium for the same amount of time as the test films were exposed to the disinfectant. The virus films were individually scraped with a plastic cell scraper and passed through individual Sephadex columns in the same manner as the test virus. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity.

8. BTC 835 Validation Control Substance

Two dried virus films were exposed to 2.0 mL of each concentration of the BTC 835 control substance (175 ppm and 350 ppm) for ten minutes at 20.1°C once per day of testing. Following the exposure time, each plate was individually scraped with a cell scraper to resuspend the contents and the virus-control substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

9. Cytotoxicity Assay

A 2.0 mL aliquot of the use dilution of the test substance and each concentration of the BTC 835 control substance were filtered through individual Sephadex columns utilizing the syringe plunger. The filtrates were titered by 10-fold serial dilution and assayed for cytotoxicity. Cytotoxicity of the duck hepatocytes was scored at the same time as virus-disinfectant and virus control cultures.

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 11 of 16



10. Assay of Non-Virucidal Level of Test Substance – (Neutralization Control)

Each dilution of the Sephadex-filtered disinfectant (disinfectant control for cytotoxicity assay) and each concentration of the BTC 835 control substance were mixed with an aliquot of low titer stock virus. The resulting mixtures of dilutions were assayed for infectivity in order to determine the dilution(s) of disinfectant and control 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.

11. Infectivity Assays

Primary duck hepatocytes were used as the indicator cell line in the infectivity assays. Cells contained in cell culture labware were inoculated in quadruplicate with 1.0 mL of the dilutions prepared from the virus control, test, and validation control substances. The cytotoxicity and neutralization control dilutions were inoculated in duplicate. A 2.0 mL aliquot of test medium was added to each test and control well in addition to the inoculum. Uninfected indicator cell cultures (negative cell controls) were inoculated with test medium alone. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO₂. Following the adsorption period, a 3.0 mL aliquot of test medium was added to each cell culture well. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ for ten days. The test medium was aspirated from each test and control well and replaced with 4.0 mL of fresh medium as needed throughout the incubation period. On the final day of incubation, the cultures were observed microscopically for test substance cytotoxicity and the cells were then fixed with ethanol. An indirect immunofluorescence assay was then performed using a monoclonal antibody specific for the envelope protein of the DHBV.

STUDY ACCEPTANCE CRITERIA

A valid test requires 1) that at least 4 log₁₀ of infectivity be recovered from the dried virus control films; 2) that when cytotoxicity is evident, at least a 3-log reduction in viral titer is demonstrated beyond the cytotoxic level; and 3) that the cell controls be negative for infectivity. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions and demonstrate a 3-log reduction in virus titer.

Project No. A02070
 Protocol Number: SRC27022304.DHBV.2

Virox Technologies
 Page 12 of 16



PROTOCOL CHANGES

Protocol Amendments

The protocol was amended for the following reasons:

- 1) To change the location for the perfusion of the duck livers. The liver perfusions will be performed at the AAALAC accredited Minneapolis Veterinary Medical Unit Services located at the Department of Veterans Affairs Medical Center, Minneapolis, MN by properly trained personnel.
- 2) To change the Sponsor's address to their current address:
 Virox Technologies
 2815 Bristol Circle, Unit 4
 Oakville, Ontario L6H6X5
- 3) To update the proposed start date to August 13, 2004 and the proposed completion date to September 10, 2004.

Protocol Deviations:

No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

Viral and cytotoxicity titers are expressed as $-\log_{10}$ of the 50 percent titration endpoint for infectivity (TCID₅₀) or cytotoxicity (TCD₅₀), respectively, as calculated by the method of Spearman Karber.

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

Statistical Analysis

The \log_{10} reduction in infectivity was calculated using the EPA approved method for calculating the Most Probable Number (MPN) as obtained by the EPA on January 4, 2001.

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 13 of 16



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 on Water and Environmental Microbiology, 2000, E1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces.
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. Environmental Protection Agency Federal Register: August 25, 2000 (Volume 65, Number 166).
7. Statistical Analysis of Hepatitis B Carrier Test Data (Revised 1-01) Template for Calculating the Log Reduction (LR) and Associated Standard Error (SE). M. Hamilton, Center for Biofilm Engineering, Montana State University. January 9, 2001, Published January 4, 2001

Project No. A02070
Protocol Number: SRC27022304.DHBV.2

Virox Technologies
Page 14 of 16



STUDY RESULTS

Results of the test with one lot of ACCEL TB (Lot 2-3646-REG-US), ready to use, exposed to duck Hepatitis B virus at 20.1°C for one minute in the presence of 100% duck serum soil load, are shown in Tables 1 and 2. The input titer (not dried) of the virus was 5.5 log₁₀. The titer of the dried virus control was 5.5 log₁₀ for both replicates. The MPN for both dried virus control replicates is 239791. Following exposure, test virus infectivity was not detected in either replicate of the virus-test substance mixture at any dilution tested (≤ 1.5 log₁₀). The MPN for both test replicates is ≤ 23.979 . Test substance cytotoxicity was not observed at any dilution tested (≤ 1.5 log₁₀). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤ 1.5 log₁₀.

Utilizing the statistical program provided by the EPA, the log reduction in viral titer is ≥ 4.00 and the standard error of the log reduction is 0.00.

Results of the test with two concentrations of BTC 835 are shown in Tables 3 and 4. Following the ten minute exposure time, test virus infectivity was detected in the virus - BTC 835 mixture in both replicates of the 175 ppm concentration at ≥ 4.5 log₁₀ and at 3.75 log₁₀ in both replicates of the 350 ppm concentration. Test substance cytotoxicity was not observed at any dilution tested (≤ 1.5 log₁₀) in either concentration of the BTC 835 control substance. The neutralization control (non-virucidal level of the control substance) indicates that both concentrations of the BTC 835 control substance were neutralized at ≤ 1.5 log₁₀.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 100% duck serum soil load, ACCEL TB (Lot 2-3646-REG-US), ready to use, demonstrated complete inactivation of duck Hepatitis B virus following a one minute exposure time at 20.1°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 affected the quality or integrity of the data.

The use of the ATS Labs name, logo or any other representation of ATS Labs, other than distribution of this report in its entirety, 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.

Project No. A02070
 Protocol Number: SRC27022304.DHBV.2

Virox Technologies
 Page 15 of 16



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

Dilution	Input Virus Control	Dried Virus Control		DHBV+ Lot 2-3646-REG-US	
		Replicate #1	Replicate #2	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻²	NT	NT	NT	0 0 0 0	0 0 0 0
10 ⁻³	NT	NT	NT	0 0 0 0	0 0 0 0
10 ⁻⁴	++++	++++	++++	0 0 0 0	0 0 0 0
10 ⁻⁵	++++	++++	++++	NT	NT
10 ⁻⁶	0 0 0 0	0 0 0 0	0 0 0 0	NT	NT
10 ⁻⁷	0 0 0 0	0 0 0 0	0 0 0 0	NT	NT
TCID ₅₀ /1.0 mL	10 ^{5.5}	10 ^{5.5}	10 ^{5.5}	≤10 ^{1.5}	≤10 ^{1.5}
MPN	NA	239791	239791	≤23.979	≤23.979
Log ₁₀ MPN	NA	5.37983	5.37983	≤1.37983	≤1.37983
Log Reduction	NA	NA		≥4.00	

TABLE 2: Cytotoxicity and Neutralization Controls of ACCEL TB

Dilution	Cytotoxicity Control Lot 2-3646-REG-US	Neutralization Control Lot 2-3646-REG-US
Cell Control	0 0	0 0
10 ⁻²	0 0	++
10 ⁻³	0 0	++
TCD ₅₀ /1.0 mL	≤10 ^{1.5}	See below

(+) = Positive for the presence of test virus
 (0) = No test virus recovered and/or no cytotoxicity present
 (NT) = Not Tested
 (MPN) = Most Probable Number
 (NA) = Not applicable

The results of the neutralization control indicate the test substance was neutralized at the dilution equivalent to ≤1.5 log₁₀ TCID₅₀/1.0 mL as compared to the treated test samples.

Project No. A02070
 Protocol Number: SRC27022304.DHBV.2

Virox Technologies
 Page 16 of 16



TABLE 3: Effects of BTC 835 Following a Ten Minute Exposure to DHBV Dried on an Inanimate Surface

Dilution	DHBV+ 175 ppm BTC 835		DHBV+ 350 ppm BTC 835	
	Replicate #1	Replicate #2	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻²	++++	++++	++++	++++
10 ⁻³	++++	++++	++++	++++
10 ⁻⁴	++++	++++	0 + 0 0	0 0 0 +
TCID ₅₀ /1.0 mL	≥10 ^{4.5}	≥10 ^{4.5}	10 ^{3.75}	10 ^{3.75}

TABLE 4: Cytotoxicity and Neutralization Controls of BTC 835

Dilution	Cytotoxicity Control 175 ppm BTC 835	Cytotoxicity Control 350 ppm BTC 835	Neutralization Control 175 ppm BTC 835	Neutralization Control 350 ppm BTC 835
Cell Control	0 0	0 0	0 0	0 0
10 ⁻²	0 0	0 0	++	++
10 ⁻³	0 0	0 0	++	++
TCD ₅₀ /1.0mL	≤10 ^{1.5}	≤10 ^{1.5}	See below	See below

(+) = Positive for the presence of test virus

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

Results of the neutralization control indicate that both concentrations of the BTC 835 control substance were neutralized at the dilution equivalent to ≤1.5 log₁₀ TCID₅₀/1.0 mL as compared to the treated BTC 835 samples.