



FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces – Confirmatory Assay

Virus: Bovine Viral Diarrhea virus as a surrogate for Human Hepatitis C virus

PRODUCT IDENTITY

ACCEL TB
Lot 2-3646-REG-US

DATA REQUIREMENT

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

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 L6H 6X5

PROJECT NUMBER

A02364

Page 1 of 16

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

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: 10/11/04

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

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: 10/11/04

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. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 4 of 16

QUALITY ASSURANCE UNIT SUMMARY

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

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. This study was inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	August 23, 2004	August 23, 2004	September 9, 2004
Draft Report	September 9, 2004	September 9, 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. Edinsson Date: 09/30/04

Project No. A02364
 Protocol Number: SRC27060204.BVD.1

Virox Technologies
 Page 5 of 16



TABLE OF CONTENTS

Title Page	1
Statement of No Data Confidentiality Claims	2
Good Laboratory Practice Statement.....	3
Quality Assurance Unit Summary	4
Table of Contents	5
Study Personnel	6
General Study Information.....	7
Test Substance Identity	7
Study Dates	7
Objective	7
Summary of Results	8
Test System	8
Test Method	9
Study Acceptance Criteria	12
Protocol Changes	12
Data Analysis.....	12
Study Retention	13
References	13
Study Results	14
Study Conclusion.....	14
Table 1: Virus Controls and Test Substance Assay Results	15
Table 2: Test Substance Cytotoxicity and Neutralization Control Results	15
Table 3: Bardac 2280 Assay Results	16
Table 4: Bardac 2280 Cytotoxicity And Neutralization Control Results	16

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

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

Project No. A02364
Protocol Number: SRC27060204.BVD.1

Virox Technologies
Page 7 of 16



STUDY REPORT

GENERAL STUDY INFORMATION

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

Project Number: A02364

Protocol Number: SRC27060204.BVD.1

Sponsor: Virox Technologies
2815 Bristol Circle, Unit 4
Oakville, Ontario L6H 6X5

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

TEST SUBSTANCE IDENTITY

Test Substance Name: ACCEL TB

Lot/Batch: 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 Bardac 2280 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: August 11, 2004
Experimental Start Date: August 19, 2004
Experimental End Date: August 26, 2004
Study Completion Date: September 30, 2004

OBJECTIVE

The purpose of this study was to evaluate the virucidal efficacy of a disinfectant against Bovine Viral Diarrhea virus as a surrogate for Human Hepatitis C virus according to test criteria and methods approved by the U.S. Environmental Protection Agency for registration of a product as a virucide.

Human Hepatitis C virus (HCV), a member of the Flaviviridae Family of enveloped RNA-containing viruses, presents a serious public safety concern. However, at present, there is no reliable *in vitro* infectivity assay for this virus. Bovine Viral Diarrhea virus, also a member of Flaviviridae Family, serves as a valuable model virus for Human Hepatitis C virus, since these viruses share many similar characteristics.

Project No. A02364
Protocol Number: SRC27060204.BVD.1

Virox Technologies
Page 8 of 16



SUMMARY OF RESULTS

Test Substance: ACCEL TB, Lot 2-3646-REG-US
Dilution: Ready to use (RTU)
Virus: Bovine Viral Diarrhea virus
Exposure Time: One minute
Exposure Temperature: 20±1°C
Organic Soil Load: 5% horse serum
Efficacy Result: One lot of ACCEL TB (Lot 2-3646-REG-US) met the test criteria specified in the study protocol. The results indicate **complete inactivation** of Bovine Viral Diarrhea virus under these test conditions.

TEST SYSTEM

1. Virus

The NADL strain of Bovine Viral Diarrhea virus (BVDV) used for this study was obtained from ViroMed Laboratories, Camden, NJ. ViroMed Laboratories received the virus from The National Veterinary Services Laboratories (NVSL). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 1800 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, three aliquots of the stock virus (ATS Labs Lot BVDC-19) were removed, thawed, combined and refrigerated until use in the assay. The stock virus culture was adjusted to contain 5% horse serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Bovine Viral Diarrhea virus on bovine turbinate cells. Cells exhibiting CPE were rounded, dark, with a granular appearance.

2. Indicator Cell Cultures

Cultures of bovine turbinate (BT) cells were originally obtained from the American Type Culture Collection (ATCC CRL-1390). The cells were propagated at ATS Labs. The cultures were maintained and used at the appropriate density in tissue culture labware at 36-38°C in a humidified atmosphere of 5-7% CO₂. This cell line has historically been used as the cell line for propagation and detection of BVDV. The cultures are commercially available, can be serially propagated, and are capable of showing cytopathic effect in the presence of the virus. The virus is also capable of propagation in this cell line to titers suitable for efficacy testing. The use of BT cells is a standard industry practice for propagation and detection of BVDV.

All cell culture documentation was retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

3. Test Medium

The test medium used for this assay was Minimal Essential Medium (MEM) supplemented with 2% (v/v) non-heat inactivated horse serum. The medium was also supplemented with the following: 10 µg/mL gentamicin, 100 units/mL penicillin and 2.5 µg/mL amphotericin B (Fungizone).

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 9 of 16

The following table outlines the specific parameters that were tested:

SAMPLES TESTED FOR THE PRESENCE OF VIRUS			
Test or Control Group	Dilutions Assayed Per Carrier (log₁₀)	Cultures (Replicates) per Dilution	Total Cultures (Replicates) Inoculated
Negative Controls	N/A	2-4	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 - Batch #1 (performed in duplicate)	-1,-2,-3,-4	4	32
Cytotoxicity Control - Batch #1	-1,-2,-3	2	6
Neutralization Control - Batch #1	-1,-2,-3	2	6
50 ppm Bardac 2280 Validation Control (performed in duplicate)	-1,-2,-3,-4	4	32
350 ppm Bardac 2280 Validation Control (performed in duplicate)	-1,-2,-3,-4	4	32
Cytotoxicity Control - 50 ppm and 350 ppm Bardac 2280	-1,-2,-3	2	12
Neutralization Control - 50 ppm and 350 ppm Bardac 2280	-1,-2,-3	2	12

TEST METHOD

1. Preparation of Test Substance

One batch of ACCEL TB (Lot 2-3646-REG-US) was used as it was received from the Sponsor. The test substance was applied according to the use directions provided by Sponsor. (See section on Treatment of Virus Films with Test Substance)

2. Preparation of Bardac 2280 Validation Control

Bardac 2280 (EPA Registration # 6836-53) received from Lonzagroup, Fair Lawn, NJ was utilized as the validation control substance. A 50 parts per million (ppm) and a 350 ppm concentration of the control substance were made in sterile deionized water.

According to the certificate of analysis supplied with the control substance, the stock Bardac 2280 is a 80.56% concentration (805,600 ppm). From this stock solution, a 1:100 dilution of the stock Bardac 2280 was made by adding 1.0 mL of Bardac 2280 to a 100 mL volumetric flask and adding sterile deionized water to the 100 mL mark, which equals a 8056 ppm concentration. The 350 ppm concentration of Bardac 2280 used in the study was made by adding 2.0 mL of the 8056 ppm concentration of Bardac 2280 to 44.04 mL sterile deionized water. The 50 ppm concentration was prepared by adding 5.0 mL of the 350 ppm concentration of Bardac 2280 to 30.0 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. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 10 of 16

On the day of testing, the total quaternary ammonium chloride (halide) concentration, of both dilutions of the Bardac 2280 validation control (50 ppm and 350 ppm), was determined using the LaMotte QAC Test Kit Model QT-DR, Code 3043-DR. The average total quaternary ammonium chloride (halide) concentration for the 50 ppm dilution was 50 ppm and the total quaternary ammonium chloride (halide) concentration for the 350 ppm dilution was 349 ppm.

3. Preparation of Virus Films

Films of virus were prepared by spreading a 0.2 mL aliquot of virus inoculum uniformly over the bottom of eight 100 X 15mm sterile glass petri dishes. The virus films were dried at 20.2°C in a relative humidity of 40.1% until visibly dry (30 minutes).

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 dried virus films were exposed to 2.0 mL of the use dilution of the test substance for the Sponsor specified one minute exposure time at 20.2°C. Following the exposure time, each plate was individually scraped with a cell scraper to resuspend the contents and the virus-test substance mixture was immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. This initial dilution was considered the 10⁻¹ dilution. A 0.2 mL aliquot of the test virus (the virus film) was resuspended in 2.0 mL of test substance which equals a 1:10 dilution. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

7. Treatment of Virus Control Films

Two virus films were prepared as described above. The virus control films were run in parallel to the test virus but 2.0 mL of test medium was added in lieu of the test substance. The control films were exposed to the test medium for the same exposure time that the test films were exposed to the test substance. The virus control films were scraped as previously described and the mixtures were immediately passed through individual Sephadex columns. The filtrates were then titered by 10-fold serial dilution and assayed for infectivity.

8. Bardac 2280 Validation Control Substance

Two dried virus films were exposed to 2.0 mL of each concentration of the Bardac 2280 control substance for ten minutes at 20.2°C. 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.

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 11 of 16

9. Cytotoxicity Assay

A 2.0 mL aliquot of the use dilution of the test substance and each concentration of the Bardac 2280 control substance was 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 BT cell cultures was scored at the same time as virus-disinfectant and virus control cultures.

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

Each dilution of the Sephadex-filtered disinfectant and validation control substance (cytotoxicity control dilutions) was mixed with an aliquot of low titer stock virus. The resulting mixtures of dilutions were assayed for infectivity and/or cytotoxicity 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 reduction in infectivity by the disinfectant.

11. Infectivity Assays

The BT cell line, which exhibits cytopathic effect (CPE) in the presence of Bovine Viral Diarrhea virus, was used as the indicator cell line in the infectivity assays. Cells contained in cell culture labware were inoculated in quadruplicate with a 0.1 mL aliquot of the dilutions prepared from the input virus control, the dried virus control, test, and validation control substances. The cytotoxicity and neutralization control dilutions were inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with a 1.0 mL aliquot of test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ in sterile disposable cell culture labware. The cultures were microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability. All cultures (cells) were examined and the results recorded. The characteristics of all cells were reported.

The determination of CPE can be subjective, therefore to verify the CPE reading, on the final day of incubation a direct immunofluorescence assay (DFA) which is a more sensitive detection method to detect the virus in the host cells was performed using a polyclonal fluorescein conjugated antibody specific for BVDV, received from VMRD, Inc., Pullman, WA. The DFA was performed only on the first inoculated dilution of the test (10⁻¹ dilution) and a dilution of the dried virus control exhibiting CPE (10⁻⁴ dilution). A negative control (cell control) was stained as well.

The DFA was performed by first aspirating the medium from each cell culture well and rinsing the cells with a 1.0 mL aliquot of phosphate buffered saline (PBS) per well. The PBS was aspirated and 1.0 mL of chilled ethanol was added to each well to fix the cells for two hours at 2-8°C. The cells were rinsed twice with approximately 1.0 mL of PBS per well. A 0.2 mL aliquot of undiluted fluorescein conjugated antibody was added to each well. The cultures were incubated in a humidified atmosphere of 36-38°C for 30 minutes. Following incubation, the cells were rinsed twice with approximately 1.0 mL of PBS per well. The PBS was removed, the plates are blotted to remove excess PBS and then observed microscopically using ultraviolet light for infectivity. A cell which was positive for the test virus fluoresced, cells negative for the test virus did not fluoresce.

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 12 of 16

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.

PROTOCOL CHANGES

Protocol Amendments:

No protocol amendments were required for this study.

Protocol Deviations:

No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

Viral and cytotoxicity titers are expressed as -log₁₀ 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 Methods

The log₁₀ reduction in infectivity is calculated using the revised EPA approved method for calculating the Most Probable Number (MPN) as obtained from the EPA on January 4, 2001.

Project No. A02364
Protocol Number: SRC27060204.BVD.1

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. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments/deviation notifications.
4. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
5. All measured data used in formulating the final report.
6. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in 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. 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. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

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 Bovine Viral Diarrhea virus in the presence of a 5% horse serum soil load at 20.2°C for one minute are shown in Tables 1 and 2. The input titer (not dried) of the virus was 5.0 log₁₀. The titer of the dried virus control was 4.75 log₁₀ for Replicate #1 and 4.5 log₁₀ for Replicate #2. The MPN of the two virus control replicates is 36164 and 23979, respectively. Following exposure, test virus infectivity was not detected in either replicate of the virus-test substance mixture at any dilution tested (≤ 0.5 log₁₀). The MPN for both test replicates is < 1.000 . Test substance cytotoxicity was not observed at any dilution tested (≤ 0.5 log₁₀). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤ 0.5 log₁₀.

The 10⁻⁴ dilution of the dried virus control was positive for test virus as determined by cytopathic effect and confirmed positive for the test virus as demonstrated by the fluorescence detected following the DFA. The negative control (cell control) and the 10⁻¹ dilution of the test were negative for the test virus by both cytopathic effect and DFA.

Utilizing the statistical program provided by the EPA, the log reduction in viral titer is ≥ 4.47 and the standard error of the log reduction is 0.09.

Results of the test with two concentrations of Bardac 2280 are shown in Tables 3 and 4. Following the ten minute exposure time, test virus infectivity was detected in the virus/Bardac 2280 mixture in both replicates of the 50 ppm concentration at 2.0 log₁₀. Following the ten minute exposure time, test virus infectivity was not detected in the virus/Bardac 2280 mixture in either replicate of the 350 ppm concentration at any dilution tested (≤ 0.5 log₁₀). Control substance cytotoxicity was not observed for either concentration at any dilution tested (≤ 0.5 log₁₀). The neutralization control (non-virucidal level of the control substance) indicates that both concentrations of the Bardac 2280 control substance were neutralized at ≤ 0.5 log₁₀.

Both replicates of the 10⁻¹ dilution of the 50 ppm concentration of Bardac 2280 were positive for the test virus as determined by cytopathic effect and confirmed positive for the test virus as demonstrated by the fluorescence detected following the DFA. Both replicates of the 10⁻¹ dilution of the 350 ppm concentration of Bardac 2280 were negative for the presence of test virus as determined by cytopathic effect and confirmed negative for the presence of test virus as demonstrated by the lack of fluorescence detected following the DFA.

STUDY CONCLUSION

Under the conditions of this investigation, in the presence of a 5% horse serum soil load, ACCEL TB (Lot 2-3646-REG-US), ready to use, demonstrated complete inactivation of Bovine Viral Diarrhea virus following a one minute exposure time at 20.2°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 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. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 15 of 16

TABLE 1: Virus Controls and Test Substance Assay Results

Effects of ACCEL TB (Lot 2-3646-REG-US) Following a One Minute Exposure to Bovine Viral Diarrhea Virus Dried on an Inanimate Surface

Dilution	Input Virus Control	Dried Virus Control		BVDV + 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 ⁻¹	NT	NT	NT	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	++00	+000	0000	NT	NT
10 ⁻⁶	0000	0000	0000	NT	NT
10 ⁻⁷	0000	0000	0000	NT	NT
TCID ₅₀ /0.1 mL	10 ^{5.0}	10 ^{4.75}	10 ^{4.5}	≤10 ^{0.5}	≤10 ^{0.5}
MPN	NA	36164	23979	<1.000*	<1.000*
Log ₁₀ MPN	NA	4.55828	4.37983	0.00000	0.00000
MPN Log Reduction	NA	NA		≥4.47	

TABLE 2: Test Substance Cytotoxicity and Neutralization Control Results

Dilution	Cytotoxicity Control Lot 2-3646-REG-US	Neutralization Control Lot 2-3646-REG-US
Cell Control	0 0	0 0
10 ⁻¹	0 0	++
10 ⁻²	0 0	++
10 ⁻³	0 0	++
TCID ₅₀ /0.1 mL	≤10 ^{0.5}	See below

(+) = Positive for the presence of test virus

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

(NT) = Not tested

(NA) = Not applicable

(MPN) = Most Probable Number

(*) = For calculating the log reduction utilizing the MPN statistical methods, MPN values of zero are reported as <1.000

The results of the neutralization control indicate that the test substance was neutralized at the dilution equivalent to ≤0.5 log₁₀ TCID₅₀/0.1 mL as compared to the treated test samples.

Project No. A02364

Virox Technologies



Protocol Number: SRC27060204.BVD.1

Page 16 of 16

TABLE 3: Bardac 2280 Assay Results**Effects of Bardac 2280 Following a Ten Minute Exposure to BVDV Dried on an Inanimate Surface**

Dilution	BVDV+ 50 ppm Bardac 2280		BVDV+ 350 ppm Bardac 2280	
	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 ⁻¹	++++	++++	0 0 0 0	0 0 0 0
10 ⁻²	++00	0++0	0 0 0 0	0 0 0 0
10 ⁻³	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10 ⁻⁴	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
TCID ₅₀ /0.1 mL	10 ^{2.0}	10 ^{2.0}	≤10 ^{0.5}	≤10 ^{0.5}

TABLE 4: Bardac 2280 Cytotoxicity and Neutralization Control Results

Dilution	Cytotoxicity Control 50 ppm Bardac 2280	Cytotoxicity Control 350 ppm Bardac 2280	Neutralization Control 50 ppm Bardac 2280	Neutralization Control 350 ppm Bardac 2280
Cell Control	0 0	0 0	0 0	0 0
10 ⁻¹	0 0	0 0	++	++
10 ⁻²	0 0	0 0	++	++
10 ⁻³	0 0	0 0	++	++
TCD ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	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 Bardac 2280 control substance were neutralized at the dilution equivalent to ≤0.5 log₁₀ TCID₅₀/0.1 mL as compared to the treated Bardac 2280 samples.