***FINAL STUDY REPORT***

***STUDY TITLE***

# A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS

***PRODUCT IDENTITY***

Test Formulation: SNIPER® (EPA Registration Number: 71700-2-82482)
Batch #1: Lot Number 109-064-1
Batch #2: Lot Number 109-064-2

***ENVIRONMENTAL PROTECTION AGENCY DATA REQUIREMENT***

U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, OCSPP 810.2200, *Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations* (March 12, 2012)

***AUTHOR***

Volha Dzyakanava, Ph.D.
Study Director, Virologist

***STUDY COMPLETION DATE***

12/17/2014

***TESTING FACILITY***

**BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

***LABORATORY STUDY NUMBER***

140821-404.01

## 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).

Submitter: _____ Date: _____
Signature

Typed Name of Signer: _____

Typed Name of Company: _____

## GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study referenced in this report was conducted in accordance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160. The following is a detailed description of all differences between the practices used in the study and those required by 40 CFR 160:

Characterization and stability of the test formulation.

Study Director: _____ Date: 12-18-14
(Signature)

Typed Name of Signer: Volha Dzyakanava, Ph.D.

Typed Name of Laboratory: BioScience Laboratories, Inc.

Sponsor: _____ Date: _____
(Signature)

Typed Name of Signer: _____

Typed Name of Company: _____

Submitter: _____ Date: _____
(Signature)

Typed Name of Signer: _____

Typed Name of Company: _____

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## QUALITY ASSURANCE STATEMENT

Study Title: **A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS**

Study No.: 140821-404.01

In accordance with the Good Laboratory Practice Standards (EPA 40 CFR § 160), quality assurance audits of this study were conducted and reported to Management and the Study Director, as listed below:

| Phase Audited | Audit Date | Date reported to Study Director | Date reported to Management |
|---|---|---|---|
| Product Testing | 11/12/2014 | 11/12/2014 | 11/14/2014 |
| Data Audit | 12/15/2014 | 12/15/2014 | 12/18/2014 |
| Final Report Review | 12/15/2014 | 12/15/2014 | 12/18/2014 |
| Amended Final Report Review | 12/18/2014 | 12/18/2014 | 12/18/2014 |

Amy Juhnke
(Signature)

Date: 12/18/14

Typed Name of Signer: Amy L. Juhnke, RQAP-GLP

QA Title: Manager of Quality Assurance

## STUDY PERSONNEL

STUDY DIRECTOR:

Volha Dzyakanava, Ph.D.
Virologist, Manager of Virology Laboratory

TEST FACILITY MANAGEMENT:

Daryl S. Paulson, Ph.D.
President and CEO

LABORATORY PERSONNEL:

Brad Berg
Materials Coordinator/Purchasing Agent

Kelly Burningham
Virologist

Stephanie Cebulla
Laboratory Support Technician

Marc Charnholm
Manager of Laboratory Support

Rodolpho Guerrero
Laboratory Support Assistant

Jennifer Robinson
Laboratory Support Technician

Heather Watkins
Microbiologist

Jessica Wells
Product Handler

QUALITY ASSURANCE PERSONNEL:

Tom Bailly
Systems Analyst

Mary Bateson, M.S.
Quality Assurance Specialist

Danielle Goveia
Quality Assurance Specialist

Aaron Hoffman
QC/Maintenance Specialist

Amy L. Juhnke, RQAP-GLP
Manager of Quality Assurance

Kim Potter
Quality Assurance Associate

Carl Schmidt
Lead Quality Control Specialist

## STUDY REPORT

**STUDY TITLE:** **A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS**

**SPONSOR:** **GLOBAL ENVIRONMENTAL RESTORATION, INC.**
PO Box 667
Carencro, Louisiana 70520

**TESTING LABORATORY:** **BIOSCIENCE LABORATORIES, INC.**
1755 South 19th Avenue
Bozeman, Montana 59718

**LABORATORY PROJECT #:** 140821-404.01

**TEST SUBSTANCE IDENTIFICATION:**

Test Formulation: SNIPER®
(EPA Registration Number 71700-2-82482)

Active Ingredient(s): Chlorine Dioxide

Batch #1
Lot Number: 109-064-1
Manufacture Date: 10-16-2014 to 10-24-2014
Expiration Date: 10-24-2015

Batch #2
Lot Number: 109-064-2
Manufacture Date: 10-16-2014 to 10-24-2014
Expiration Date: 10-24-2015

Description of Test Substance: Clear bottle containing clear liquid.

Chemical Characterization: Verification of the identity, solubility, stability, strength, purity, and chemical composition was not performed by the Testing Facility and remained the responsibility of the Sponsor.

**STUDY INITIATION DATE:** 10/27/2014

**EXPERIMENTAL START DATE:** 11/12/2014

**EXPERIMENTAL END DATE:** 11/21/2014

**STUDY COMPLETION DATE:** 12/17/2014

**STUDY OBJECTIVE:** To determine hard surface disinfection efficacy of two batches of one spray test formulation when challenged with Canine Parvovirus.

**TEST METHOD:** Testing was designed to simulate consumer use and was based upon methods described in the American Society for Test Materials (ASTM) E1053-11, *Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surface,* with modifications based on U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, OCSPP 810.2200, *Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations* (March 12, 2012) and the Official Methods of Analysis, 961.02, *AOAC Germicidal Spray Products as Disinfectants.*

**TEST SYSTEM/STRAINS:** Canine Parvovirus strain Cornell-780916 (ATCC #VR-953)
Test Date: 11-12-14
Source: American Type Culture Collection

**TEST DILUTION:** Undiluted

**EXPOSURE TIME:** 10 minutes

**EXPOSURE TEMPERATURE:** Ambient room temperature (21.5 °C - 22 °C)

**ORGANIC SOIL LOAD:** ≥5% Fetal Bovine Serum (FBS)

**HARD WATER:** Not Applicable

## STUDY MATERIALS

**MEDIA/REAGENTS:**

| | |
|---|---|
| Growth Medium (GM) | Eagle's Minimum Essential Medium (EMEM, ATCC) with 10% Horse Serum (ATCC), and 1% Penicillin/Streptomycin/Amphotericin B (Gibco) and 0.03 mg/mL rat tail collagen |
| Maintenance Medium (MM) | EMEM (ATCC) with 2% Horse Serum (ATCC) and and 1% Penicillin/Streptomycin/Amphotericin B (Gibco) |
| Neutralizing Medium | Dey-Engley (D/E) Broth |
| Miscellaneous | Trypsin-EDTA solution |

**EQUIPMENT/SUPPLIES:**

$CO_2$ Incubator, Temperature Range 37 °C ± 2 °C
$CO_2$ Incubator, Temperature Range 35 °C ± 2 °C
Portable Pipetters
Continuously Adjustable Pipettes
25 mL Pipette
10 mL Pipette
1 mL Pipette
Tubes 15 mL
Tubes 50 mL

Pipette Tips
Sterile Glass Petri Dishes (100 mm x 15 mm)
Cell Culture Vessels: 24-well plates
Thermometer
Inverted Compound Microscope
Laminar Flow Biological Safety Cabinets
Calibrated Minute/Second Timers
Balances
Cell Scrapers
NIST Radio-Controlled Clock

## STUDY METHOD

(REFERENCE PROTOCOL #140821-404, APPENDIX 1 OF THIS FINAL REPORT)

### PREPARATION OF TEST FORMULATION:

The Test Formulation was used as received.

### PREPARATION OF TEST SYSTEM AND STRAINS:

Cell Culture Preparation

CRFK cells (feline epithelial kidney cells [ATCC #CCL-94]) were maintained as monolayers in disposable cell culture labware and were used for testing of Canine Parvovirus. Cell monolayers were approximately 90% confluent and less than 48 hours old before inoculation with the virus. Growth Medium (GM) and Maintenance Medium (MM) were 1X Eagle's Minimum Essential Medium (EMEM) with supplements. Prior to plating, the GM was replaced by MM.

Virus Preparation

The test virus suspension used for this study originated from BSLI high titer virus stock prepared with 5% Fetal Bovine Serum (FBS). The virus was removed from a -70 °C freezer and thawed at room temperature prior to use in testing.

Preparation of Carriers

The inner bottom surfaces of sterilized glass Petri dishes (100 mm x 15 mm) were used as the carriers. One carrier per batch of the test formulation was used in testing.

Contamination of Carriers

A 0.2 mL aliquot of the prepared virus was transferred to the inner bottom surfaces of sterilized glass Petri plate carriers. A sterile cell scraper was used to spread the inoculum uniformly over the bottom of each 100 mm x 15 mm glass Petri plate carrier. The virus suspension was air-dried at ambient temperature until visibly dry.

### APPLICATION AND EXPOSURE CONDITIONS:

A spray apparatus was adjusted so that the distance between the horizontal countertop and the product nozzle was up to six inches. The test formulation was applied to each carrier by spraying three times from a distance of approximately six inches. Sufficient test formulation was applied to ensure that the carrier was thoroughly wetted. The carrier was exposed to each test batch at ambient temperature for 10 minutes, timed using a calibrated minute/second timer. Timing had commenced after the spray procedure was completed. The treated carrier was kept undisturbed for the duration of the contact time.

**TEST SYSTEM RECOVERY:**

The test system recovery procedures were as follows: after the exposure time elapsed, an 18.0 mL aliquot of the neutralizer (D/E Broth) was transferred into the Petri dishes, and sterile cell scrapers were used to remove surviving virus from the surface of the carrier. A series of 10-fold dilutions of the virus suspension were made in MM and plated in four replicates onto 24-well plates. The plates were incubated for 9 days in a $CO_2$ incubator at 35 °C ± 2 °C. The virus presence/absence or cytotoxicity was assessed using an inverted compound microscope (Cytopathic effect [CPE]).

For all phases of testing, CPE was rated as follows:

4 - Monolayer completely destroyed by the virus or because of cytotoxicity
3 - Substantial CPE due to virus; however, monolayer still present
2 - Approximately 30-40% of cell monolayer destroyed by the virus or because of cytotoxicity
1 - Approximately 10-20% of cell monolayer destroyed by the virus or because of cytotoxicity
0 - No CPE present

## PROTOCOL CHANGES

**PROTOCOL AMENDMENTS:**

No amendments to the protocol were made in the course of this evaluation.

**PROTOCOL DEVIATIONS:**

One deviation from the protocol occurred during the course of this evaluation. CRFK cell monolayers were approximately 90% confluent and less than 48 hours old before inoculation with virus. The deviation was intentional. CRFK cells were used for testing when cell monolayers were approximately 90% confluent and less than 48 hours old before inoculation due to the use of collagen additive in the Growth Medium. Collagen allows a better attachment of cell monolayer to the bottom of the plastic wells preventing premature peeling of cells from the surface thus, the use of less confluent (50%) and younger cells (less than 24 hour old) was not necessary. This deviation did not affect the study outcome.

## CONTROLS

**PREPARATION OF CONTROLS:**

Neutralization and Cytotoxicity Controls

One carrier per batch of the test formulation was used for the Neutralization and Cytotoxicity Controls.

A 0.2 mL aliquot of medium was transferred to the inner bottom surfaces of sterilized glass Petri dish carriers. A sterile cell scraper was used to spread the inoculum uniformly over the bottom of each 100 mm x 15 mm glass Petri dish carrier. The medium film was air-dried at ambient temperature until visibly dry. The test formulation was applied to each carrier by spraying three times from a distance of approximately six inches. Sufficient test formulation was applied to ensure that the carrier was thoroughly wetted. The carrier was exposed to each test batch at ambient temperature for 10 minutes, timed using a calibrated minute/second timer. Timing had commenced after the spray procedure was completed. The treated carrier was kept undisturbed for the duration of the contact time.

Following the exposure, 18.0 mL of the neutralizer (D/E Broth) was transferred into the Petri dishes, and sterile cell scrapers were used to scrape the surface of the carrier. The neutralized liquid was used for the Neutralization Control (virus added) and Cytotoxicity Control (no virus added). The Neutralization Control (4.5 mL) received a 0.5 mL aliquot of the test virus followed by exposure for at least 10 minutes to the neutralized product. Subsequent 10-fold dilutions were made in MM and plated in four replicates. The Cytotoxicity Control received no virus. The test formulation/neutralizer liquid was diluted (10-fold) in MM and plated in four replicates onto 24-well plates. The plates were incubated in a $CO_2$ incubator 35 °C ± 2 °C. The virus presence/absence or cytotoxicity was assessed using an inverted compound microscope.

Virus Control

Two carriers were used for the Virus Control.

A 0.2 mL aliquot of the virus was transferred to the inner bottom surfaces of sterilized glass Petri dish carriers. A sterile cell scraper was used to spread the inoculum uniformly over the bottom of 100 mm x 15 mm glass Petri dish carriers. The virus suspension was air-dried at ambient temperature until visibly dry. A 2.0 mL aliquot of MM was added to the carrier and exposed for 10 minutes at ambient temperature.

An 18.0 mL aliquot of the neutralizer (D/E Broth) was transferred into the carriers and a sterile cell scraper was used to remove the virus from the surface. Subsequent 10-fold dilutions were made in MM and plated in four replicates onto 24-well plates. The plates were incubated in a $CO_2$ incubator 35 °C ± 2 °C. The virus presence/absence or cytotoxicity was assessed using an inverted compound microscope.

Initial Population

A 0.5 mL aliquot of the test virus was diluted in 4.5 mL of the neutralizer (D/E Broth), and subsequent 10-fold dilutions were performed. Each dilution was plated in four replicates.

Cell Culture Control

Intact cell culture monolayers served as the control of cell culture viability. The Growth Medium was replaced by Maintenance Medium in all Cell Culture Control wells (minimum four wells).

## STUDY ACCEPTANCE CRITERIA

**STUDY REQUIREMENTS:**

Neutralization Requirement

The test formulation is considered neutralized if the differences between the test virus titer in the Initial Population and the Neutralization Control do not exceed 1 $\log_{10}$.

Test Requirements and Performance Criteria

Per U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, OCSPP 810.2200, *Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations* (March 12, 2012).

1. One surface for each of two different batches of disinfectant must be tested against recoverable virus titer of at least $10^4$ from the test surface.

2. The virus recovery should include a minimum of four determinations per each dilution in the assay system.

3. Cytotoxicity controls: The effect of the germicide on the assay system should include a minimum of four determinations per each dilution.

4. The activity of the germicide against the test virus should include a minimum of four determinations per each dilution in the assay system.

5. Any special methods used to increase the virus titer and to detoxify the residual germicide should be described.

6. The ID-50 values calculated for each assay.

7. The test results shall be reported as the reduction of the virus titer by the activity of the germicide (ID-50 of the virus control less the ID-50 of the test system), expressed as $\log_{10}$ and calculated by a statistical method.

8. For virucidal data to be acceptable, the formulation must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a $\geq 3$ $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. The calculated viral titers must be reported with the test results.

## DATA ANALYSIS

### CALCULATIONS:

Viral titers were expressed as $-\log_{10}$ of the 50% titration end point for infectivity. To calculate the viral titer, a 50% tissue culture infectious dose ($TCID_{50}$) calculation - the Quantal test (Spearman-Kärber Method) - was applied.

$$\text{Log } TCID_{50} = l - d\,(s - 0.5)$$

Where:

l = $-\log_{10}$ of the lowest dilution;
d = difference between dilution steps;
s = sum of proportions of positive wells.

The $\log_{10}$ and percent (%) of infectivity reductions were calculated as follows:

$\log_{10}$ Reduction = ($\log_{10}$ $TCID_{50}$ of the Virus Control) - ($\log_{10}$ $TCID_{50}$ of the Virucidal Test)

$$\%\ \text{Reduction} = \left[1 - \frac{TCID_{50}\ test}{TCID_{50}\ virus\ control}\right] \times 100$$

### STATISTICAL ANALYSIS:

A statistical analysis was not performed for this Study.

## STUDY RETENTION

### DATA RETENTION:

All raw data for this study, including the Final Report, will be retained in safe storage by BioScience Laboratories, Inc., at its facility at 7640 Shedhorn Drive, Bozeman, Montana, for the life of the formulation registration with the EPA, or as specified by the Sponsor.

## STUDY RESULTS (TABLES 1 AND 2)

Table 1 presents data from the Hard Surface Disinfection Test performed using 100 mm x 15 mm glass Petri dish carriers inoculated with Canine Parvovirus strain Cornell-780916 (ATCC #VR-953). The test formulation, SNIPER®, Batch #1 (Lot Number 109-064-1), reduced the infectivity of the virus by 4.125 $\log_{10}$ (>99.99%), following a 10-minute exposure. The average recovery of the Virus Control from two carriers was 6.625 $\log_{10}$, meeting the acceptance criterion. Cells in the Cell Culture Control wells were viable. The medium was free of contamination in all wells of the plate.

### TABLE 1

Test Formulation: SNIPER®, Batch #1, Lot Number 109-064-1
Virus: Canine Parvovirus (ATCC #VR-953)
Host Cell Line: CRFK Host Cell Line ATCC #CCL-94
Organic Soil Load: ≥5% Fetal Bovine Serum
Volume Plated per Well: 1.0 mL
Exposure Time: 10 minutes

| **Dilution (- $\log_{10}$)** | **Virus Control** | | **Test** | **Neutralization Control** | **Initial Population** | **Cytotoxicity Control** | **Cell Control** |
|---|---|---|---|---|---|---|---|
| | Carrier 1 | Carrier 2 | | | | | 0000 |
| -2 | NT | NT | CT | NT | NT | ++++ | |
| -3 | ++++ | ++++ | 0000 | ++++ | ++++ | 0000 | |
| -4 | ++++ | ++++ | 0000 | ++++ | ++++ | 0000 | |
| -5 | ++++ | ++++ | 0000 | ++++ | ++++ | NT | |
| -6 | ++++ | +++0 | 0000 | ++0+ | ++++ | NT | |
| -7 | 000+ | +000 | 0000 | 0000 | 000+ | NT | |
| $TCID_{50}$ ($\log_{10}$) | 6.750 | 6.500 | 2.500 | 6.250 | 6.750 | 2.500 | N/A |
| Average $TCID_{50}$ ($\log_{10}$) | 6.625 | | 2.500 | N/A | | | |
| $\log_{10}$ Reduction | N/A | | **4.125** | | | | |
| Percent Reduction | | | **>99.99** | | | | |

\+ CPE (cytopathic/cytotoxic effect) present
**0** CPE (cytopathic/cytotoxic effect) not detected
**NT** Not tested
**N/A** Not applicable
**CT** Cytotoxicity

Note: $\log_{10}$ Reduction = Average Virus Control $\log_{10}$ $TCID_{50}$ – Test Carrier $\log_{10}$ $TCID_{50}$.

Table 2 presents data from the Hard Surface Disinfection Test performed using 100 mm x 15 mm glass Petri dish carriers inoculated with Canine Parvovirus strain Cornell-780916 (ATCC #VR-953). The test formulation, SNIPER®, Batch #2 (Lot Number 109-064-2), reduced the infectivity of the virus by 4.125 $\log_{10}$ (>99.99%), following a 10-minute exposure. The average recovery of the Virus Control from two carriers was 6.625 $\log_{10}$, meeting the acceptance criterion. Cells in the Cell Culture Control wells were viable. The medium was free of contamination in all wells of the plate.

**TABLE 2**

Test Formulation: SNIPER®, Batch #2, Lot Number 109-064-2
Virus: Canine Parvovirus (ATCC #VR-953)
Host Cell Line: CRFK Host Cell Line ATCC #CCL-94
Organic Soil Load: ≥5% Fetal Bovine Serum
Volume Plated per Well: 1.0 mL
Exposure Time: 10 minutes

| **Dilution (- $\log_{10}$)** | **Virus Control** | | **Test** | **Neutralization Control** | **Initial Population** | **Cytotoxicity Control** | **Cell Control** |
|---|---|---|---|---|---|---|---|
| | Carrier 1 | Carrier 2 | | | | | 0000 |
| -2 | NT | NT | CT | NT | NT | ++++ | |
| -3 | ++++ | ++++ | 0000 | ++++ | ++++ | 0000 | |
| -4 | ++++ | ++++ | 0000 | ++++ | ++++ | 0000 | |
| -5 | ++++ | ++++ | 0000 | ++++ | ++++ | NT | |
| -6 | ++++ | +++0 | 0000 | ++++ | ++++ | NT | |
| -7 | 000+ | +000 | 0000 | +000 | 000+ | NT | |
| $TCID_{50}$ ($\log_{10}$) | 6.750 | 6.500 | 2.500 | 6.750 | 6.750 | 2.500 | N/A |
| Average $TCID_{50}$ ($\log_{10}$) | 6.625 | | 2.500 | N/A | | | |
| $\log_{10}$ Reduction | N/A | | **4.125** | | | | |
| Percent Reduction | | | **>99.99** | | | | |

| | |
|---|---|
| + | CPE (cytopathic/cytotoxic effect) present |
| **0** | CPE (cytopathic/cytotoxic effect) not detected |
| **NT** | Not tested |
| **N/A** | Not applicable |
| **CT** | Cytotoxicity |

Note: $\log_{10}$ Reduction = Average Virus Control $\log_{10}$ $TCID_{50}$ – Test Carrier $\log_{10}$ $TCID_{50}$.

**NEUTRALIZATION RESULTS**

Two batches of the test formulation were fully neutralized and did not affect the virus infectivity as the difference between the test virus titer in the Initial Population and the Neutralization Control did not exceed 1 $\log_{10}$. Initial Population $TCID_{50}$ – 6.750 $\log_{10}$; Neutralization Control for Batch #1 Lot #109-064-1 – 6.250 $\log_{10}$; Neutralization Control for Lot # 109-064-2 – 6.750 $\log_{10}$.

## STUDY CONCLUSION

Under the conditions of this evaluation, Test Formulation, SNIPER®, Batch #1 (Lot Number 109-064-1), reduced the infectivity of Canine Parvovirus (ATCC #VR-953) by 4.125 $\log_{10}$ (>99.99%). Test Formulation, SNIPER®, Batch #1 (Lot Number 109-064-1), demonstrated complete inactivation of Canine Parvovirus above the cytotoxicity level, following a 10-minute exposure.

Under the conditions of this evaluation, Test Formulation, SNIPER®, Batch #2 (Lot Number 109-064-2), reduced the infectivity of Canine Parvovirus (ATCC #VR-953) by 4.125 $\log_{10}$ (>99.99%). Test Formulation, SNIPER®, Batch #2 (Lot Number 109-064-2), demonstrated complete inactivation of Canine Parvovirus above the cytotoxicity level, following a 10-minute exposure.

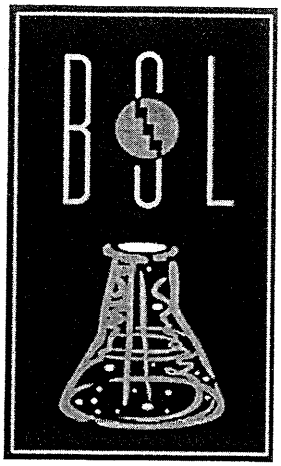
**REPORT SUBMITTED BY:**

Study Director

12-18-2014
Study Completion Date

## APPENDIX 1

Protocol #140821-404
Protocol and/or SOP Deviation Recording Form (Form No. 99-QA-004)
Final Report Amendment Form – EPA (Form No. 99-G-006)




October 14, 2014

PROTOCOL #140821-404

# A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS

Prepared for:

**GLOBAL ENVIRONMENTAL RESTORATION, INC.** (SPONSOR)
PO Box 667
Carencro, Louisiana 70520

Prepared by:

**BIOSCIENCE LABORATORIES, INC.** (TESTING FACILITY)
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October 14, 2014

PROTOCOL #140821-404

**1.0 TITLE:** A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS

**2.0 SPONSOR:** GLOBAL ENVIRONMENTAL RESTORATION, INC.
PO Box 667
Carencro, Louisiana 70520

**3.0 TESTING FACILITY:** BIOSCIENCE LABORATORIES, INC.
1755 South 19th Avenue
Bozeman, Montana 59718

**4.0 STUDY DIRECTOR:** Volha Dzyakanava, Ph.D.

**5.0 PROPOSED EXPERIMENTAL START DATE:** October, 2014

**6.0 PROPOSED EXPERIMENTAL COMPLETION DATE:** November, 2014

**7.0 PURPOSE:**

This study is designed to evaluate virucidal efficacy of two batches of one test formulation when challenged with Canine Parvovirus strain Cornell-780916 (ATCC #VR-953). Testing will be performed as described in the procedure outlined in the American Society for Test Materials (ASTM) test method designated E 1053-11, *Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces,* as specified in the U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, OCSPP 810.2200, *Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations* (March 12, 2012) an based upon the Official Methods of Analysis, 961.02, *AOAC Germicidal Spray Products as Disinfectants.* All testing will be performed in accordance with Good Laboratory Practices, as specified in 40 CFR 160, with the exception that the Study Sponsor retains responsibility for the determination of the identity, strength, purity, composition, and stability of the test formulations.

**8.0 SCOPE:**

This study will evaluate the virucidal efficacy of two batches of one spray disinfectant product, when used on dry, non-porous, inanimate surfaces. The test product will be evaluated versus Canine Parvovirus in the presence of an Organic Soil Load (OSL). A challenge suspension will be used to inoculate the bottom part of 100 mm X 15 mm glass Petri Dish carriers (one carrier per test product) to yield a minimum of $10^4$ viruses per carrier following drying. After drying, each carrier will be treated using a spray application from a distance of four to six inches until thoroughly wet (3-4 sprays) and exposed at room temperature for 10 minutes. Following the timed exposure, the neutralizer appropriate for test formulation will be added to the carrier. An aliquot of the neutralized suspension will be serially diluted in medium and assayed for the presence of viable viruses using the susceptible to the virus cell culture. The viral titers will be determined using a 50% tissue culture infectious dose ($TCID_{50}$) calculation -- the Quantal test (Spearman-Kärber Method).

## 9.0 TEST MATERIAL:

The test product to be evaluated will be provided to the Testing Facility by the Study Sponsor, complete with appropriate documentation. If the product name and/or lot number are not documented below, the information will be presented in the Final Report. Responsibility for the determination of the identity, strength, purity, composition, stability, and solubility of the test product, as well as the retention of the test product, rests with the Sponsor.

Test Product:
Active Ingredients: _____

Batch #1 _____
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

Batch #2 _____
Lot Number: _____
Manufacture Date: _____
Expiration Date: _____

## 10.0 TEST CONDITIONS:

| | | |
|---|---|---|
| 10.1 | Exposure Time: | 10 minutes |
| 10.2 | Exposure Temperature: | 22 °C ± 2 °C |

## 11.0 EQUIPMENT:

11.1 $CO_2$ Incubator, Temperature Range 37 °C ± 2 °C
11.2 $CO_2$ Incubator, Temperature Range 35 °C ± 2 °C
11.3 Incubator and Water Bath Thermometers
11.4 Portable Pipetter
11.5 Continuously Adjustable Pipettes, 100 µL – 1000 µL Capacity
11.6 Continuously Adjustable Pipettes, 20 µL – 200 µL Capacity
11.7 Inverted Compound Microscope
11.8 Laminar Flow Biological Safety Cabinet
11.9 Waste Pan
11.10 Calibrated Minute/Second Timers

## 12.0 SUPPLIES:

12.1 Sterile Disposable Pipettes
12.2 Sterile Polystyrene Test Tubes
12.3 Sterile Universal 1.0 mL and 0.2 mL Pipette Tips
12.4 Sterile, Powder-Free Gloves
12.5 Sterile Tissue Culture Treated 24-Well Plates
12.6 Viral Suspension
12.7 Sterile 100 µL and 1000 µL Displacement Tips
12.8 Sterile Flasks
12.9 Sterile 50 mL Centrifuge Tubes
12.10 Sterile Pipette Reservoir
12.11 Non-Sterile Waste Beaker for discarded tips, etc.
12.12 Sterile Cell Scrapers
12.13 Sterile Glass Petri Dishes

**13.0 MEDIA:**

13.1 1X Eagle's Minimum Essential Medium (1X EMEM)
13.2 Trypsin
13.3 Antibiotics
13.4 Product Neutralizer
13.5 Horse Serum
13.6 Fetal Bovine Serum (FBS)

**14.0 ORGANIC SOIL LOAD:**

Fetal Bovine Serum (FBS), at the final concentration of ≥ 5%.

**15.0 CHALLENGE VIRAL STRAIN:**

Canine Parvovirus strain Cornell-780916 (ATCC #VR-953).
ATCC = American Type Culture Collection

**16.0 HOST CELLS:**

CRFK (Feline kidney cells, ATCC #CCL-94).

**17.0 HOST CELL PREPARATION:**

CRFK cells (ATCC #CCL-94) will be maintained as monolayers in disposable cell culture labware and will be used for testing of Canine Parvovirus. Cell monolayers will be approximately 50% to 60% confluent and less than 24 hours old before inoculation with virus. Growth Medium (GM) and Maintenance Medium (MM) will be 1X Eagle's Minimum Essential Medium (EMEM) with appropriate supplements. Prior to plating, the GM will be replaced by MM.

**18.0 TEST VIRUS PREPARATION:**

Virus from BSLI high-titer virus stock will be used for this study. On the day of use, aliquots of the stock virus will be removed from a -70°C freezer and thawed. Prior to use in testing, FBS will be added to the test virus suspension to achieve a final concentration of ≥ 5%.

**19.0 TEST PROCEDURE:**

19.1 Preparation of Carriers

Sterilized glass Petri plates (100 mm x 15 mm) will be used as the carriers for this evaluation.

19.2 Contamination of Carriers

19.2.1 A 0.2 mL aliquot of the prepared virus suspension will be transferred to the bottom inside surface of a sterilized 100 mm x 15 mm glass Petri plate carrier. A sterile cell scraper will be used to spread the inoculum uniformly.

19.2.2 The virus suspension will be air-dried at room temperature until visibly dry.

19.2.3 One carrier will be used per batch of spray formulation.

19.3 Spray Formulation Test

19.3.1 After the inoculated carriers have dried, one carrier will be treated with each batch of the test formulation per Sponsor specifications and /or label instructions. A spray apparatus will be adjusted so that the distance between the horizontal countertop and the product nozzle is four

to six inches. The test formulation will be applied to each carrier by spraying three to four times from a distance of four to six inches. Sufficient test formulation will be applied to ensure that the carrier is thoroughly wetted. The carrier will be exposed to each test formulation at ambient temperature for 10 minutes, timed using a calibrated minute/second timer. Timing will commence after the spray procedure is completed. The treated carrier will be kept undisturbed for the duration of the contact time.

19.3.2 After the exposure time has elapsed, the appropriate amount of the neutralizer (not more than 20 mL) will be added to the Petri dish and the virus test formulation mixture will be scraped from the surface of the carrier using a sterile cell scraper. Subsequent 10-fold dilutions will be made in MM and plated in four replicates.

19.3.3 *Virus control.* Two carriers will be used for Virus Control. The test virus will be dried as described in Section 19.2.1 and 19.2.1. A total of 2.0 mL of MM will be added to the contaminated carriers. The carriers will be exposed to MM at ambient temperature for 10 minutes, timed using a calibrated minute/second timer. The appropriate neutralizer will be added to the carriers and the virus will be scraped from the surface. Subsequent 10-fold dilutions will be made in MM and plated in four replicates.

19.3.4 *Initial Population.* The test virus will be diluted in the Neutralizer, and subsequent 10-fold dilutions will be performed in MM. Each dilution will be plated in four replicates.

19.3.5 *Neutralization and Cytotoxicity Controls.* A 0.2 mL aliquot of medium will be transferred to the bottom inside surface of a sterilized 100 mm x 15 mm glass Petri plate carrier. A sterile cell scraper will be used to spread the medium uniformly. The medium will be air-dried at room temperature until visibly dry. After drying, the carrier will be treated as described in Section 19.3.1. After the exposure time has elapsed, the appropriate neutralizer will be dispensed into the carriers. The neutralized liquid will be used for the Neutralization control (virus added) and Cytotoxicity Control (no virus added). The Neutralization Control will receive an aliquot of the test virus, followed by exposure for at least 10 minutes, subsequent 10-fold dilutions in MM, and plating in four replicates. The Cytotoxicity Control will receive no virus, will be diluted (10-fold) in MM, and plated in four replicates.

19.3.6 *Cell Culture Control.* Intact cell culture monolayers will serve as the control of cell culture viability. The Growth Medium will be replaced by MM in all cell culture control wells (minimum four wells).

19.3.7 The plates will be incubated for 5 to 14 days at 35 °C ± 2 °C in a $CO_2$ incubator. Cytopathic/ cytotoxic effect will be monitored using an inverted compound microscope.

## 20.0 CALCULATIONS:

20.1 Viral titers will be expressed as -$Log_{10}$ of the 50% titration end point for infectivity. To calculate the viral titer, a 50% tissue culture infectious dose ($TCID_{50}$) calculation - the Quantal test (Spearman-Kärber Method) - will be applied.

$$\text{Log } TCID_{50} = L - d\,(s - 0.5)$$

Where:
L = $-Log_{10}$ of the lowest dilution;
d = difference between dilution steps;
s = sum of proportions of positive wells.

20.2 The $Log_{10}$ and percent (%) of infectivity reductions will be calculated as follows:

$$\%\ \text{Reduction} = \left[1 - \frac{TCID_{50}\ test}{TCID_{50}\ virus\ control}\right] \times 100$$

$Log_{10}$ Reduction = ($Log_{10}$ $TCID_{50}$ of the Virus Control) - ($Log_{10}$ $TCID_{50}$ of the Virucidal Test)

## 21.0 TEST ACCEPTANCE CRITERIA:

A valid test requires: 1) at least 4 $log_{10}$ of $TCID_{50}$ be recovered from the Virus control; 2) cells in the Negative control wells be viable and attached to the bottom of the well; 3) the medium be free of contamination in all wells of the plate; 4) when cytotoxicity is evident, at least a 3 $Log_{10}$ reduction in titer be demonstrated beyond the cytotoxic level; 5) the test formulation be fully neutralized, so the difference between the test virus titer in Initial Population and Neutralization Control does not exceed 1.0 $log_{10}$.

## 22.0 STATISTICAL ANALYSIS:

A statistical analysis will not be performed on the data derived from this evaluation.

## 23.0 FINAL REPORT:

A Final Report will be issued that presents the results in a clear and concise manner.

## 24.0 EXCEPTIONAL CONDITIONS:

The Sponsor will be notified by telephone, email, and/or letter of any exceptions encountered in this study. The exceptional conditions or occurrences will be detailed in full and formally recorded. Exceptional conditions that occur and are not addressed in this Protocol will be subject to Out-of-Scope charges (See Proposal/Contract).

## 25.0 LIABILITY AND INDEMNIFICATION:

The Testing Facility's liability to the Study Sponsor under this Protocol shall be limited to the price of this evaluation. The Study Sponsor shall be responsible to Study Participants (when applicable) and to other third parties for the fitness of the product for use as defined in the Study Protocol.

## 26.0 REFERENCES:

26.1 ASTM E1053-11, *Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces.*

26.2 U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, OCSPP,

810.220: *Disinfectants for Use on Hard Surfaces-Efficacy Data Recommendations* (March 12, 2012).

26.3 Official Methods of Analysis of AOAC International, Official Method 961.02, *AOAC Germicidal Spray Products as Disinfectants.*

## 27.0 DOCUMENTATION AND RECORD-KEEPING:

All documentation and records will be compiled, analyzed, and retained by BioScience Laboratories, Inc., at its facility in Bozeman, Montana. All raw data for this study will be retained in safe storage for the life of the product registration with the EPA, or as specified by the Sponsor.

## 28.0 PRODUCT DISPOSITION:

It is the responsibility of the Sponsor to retain a sample of the test substance(s) for future audit or evaluation. All unused test material will be disposed of following study completion, unless otherwise indicated by the Sponsor prior to initiation of the study.

## 29.0 QUALITY ASSURANCE AUDITS:

The Quality Assurance Unit (QAU) will conduct in-phase audits of critical processes in testing at least once and advise the Study Director and Management of the outcomes of these. On completion of testing, the QAU will perform an audit of the data and of the Final Report in its entirety.

30.0 **ACCEPTANCE:**

**A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS**

**ACCEPTED BY: BIOSCIENCE LABORATORIES, INC.** (TESTING FACILITY)
1755 South 19th Avenue
Bozeman, Montana 59718

Study Director: _____ 10-27-14
Volha Dzyakanava, Ph.D. — Date of Study Initiation

**ACCEPTED BY: GLOBAL ENVIRONMENTAL RESTORATION, INC.** (SPONSOR)
PO Box 667
Carencro, Louisiana 70520

Alan Campbell — 10-27-14
Representative — Date

President
Title

## PROTOCOL AND/OR SOP DEVIATION RECORDING FORM

**DATE:** 11/21/2014

**DEVIATION NUMBER:** 01

**PROTOCOL NUMBER:** 140821-404

**SOP NUMBER:** N/A

**SPONSOR:** Global Environmental Restoration

**RECORDED BY:** Volha Dzyakanava

**PROTOCOL TITLE: A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS**

**PROCEDURE AS OUTLINED IN THE PROTOCOL AND/OR SOP:** The Protocol Section 17.0 "CELL CULTURE PREPARATION" states: CRFK cells (ATCC #CCL-94) will be maintained as monolayers in disposable cell culture labware and will be used for testing of Canine Parvovirus. Cell monolayers will be approximately 50% to 60% confluent and less than 24 hours old before inoculation with virus.

**DEVIATION FROM PROCEDURE:** CRFK cells (ATCC #CCL-94) were maintained as monolayers in disposable cell culture labware and were used for testing of Canine Parvovirus. Cell monolayers were approximately 90% confluent and less than 48 hours old before inoculation with virus.

**TYPE OF DEVIATION (CHECK ONE):**

☑ **Protocol** ☐ **Measurement** ☐ **Material** ☐ **Personnel** ☐ **Environmental**
☐ **Methods** ☐ **Equipment/Instrumentation** ☐ **Subject Recruitment** ☐ **Other**

**REASON FOR DEVIATION AND EFFECT ON STUDY OUTCOME:**
The deviation was intentional. CRFK cells were used for testing when cell monolayers were approximately 90% confluent and less than 48 hours old before inoculation due to the use of collagen additive in the Growth Medium. Collagen allows a better attachment of cell monolayer to the bottom of the plastic wells preventing premature peeling of cells from the surface thus, the use of less confluent (50%) and younger cells (less than 24 hour old) was not necessary. This deviation did not affect the study outcome.

**ACKNOWLEDGMENTS:**

| | |
|---|---|
| STUDY DIRECTOR | 11-21-14 DATE |
| STUDY MANAGEMENT | 11/25/14 DATE |
| CORPORATE MANAGEMENT | 11-25-14 DATE |
| QUALITY ASSURANCE | 11/21/14 DATE |

**ASSESSMENT OF NONCONFORMANCE (CHECK ONE):**
☐ **Critical** ☐ **Major** ☑ **Minor**

FINAL REPORT AMENDMENT FORM - EPA

**DATE:** 12/18/2014 **AMENDMENT NUMBER: 01**

**STUDY NUMBER:** 140821-404

**SPONSOR:** GLOBAL ENVIRONMENTAL RESTORATION, INC.

**FINAL REPORT TITLE:** A GLP HARD SURFACE DISINFECTION EVALUATION OF ONE TEST PRODUCT VERSUS CANINE PARVOVIRUS

**REASON(S) FOR AMENDMENT:** The Sponsor has provided the EPA Registration Number and requested it to be added to the Final Report.

The revised Final Report will state: SNIPER® (EPA Registration Number: 71700-2-82482)
Batch #1: Lot Number 109-064-1
Batch #2: Lot Number 109-064-2

An amended final report, Final Report #140821-404.01, will be issued and is intended to replace Final Report #140821-404, issued on December 17, 2014, in its entirely.

**APPROVALS:**

Marsha Paulson — CORPORATE MANAGEMENT — 12/18/14 — DATE

STUDY DIRECTOR — 12-18-2014 — DATE

Alan Campbell — SPONSOR — 12/18/2014 — DATE

**REVIEWED BY:**

QUALITY ASSURANCE — 12/18/14 — DATE

## APPENDIX 2

Transmittal of Proprietary Rights

## TRANSMITTAL OF PROPRIETARY RIGHTS

**TO:** Global Environmental Restoration, Inc.

**FROM:** Daryl S. Paulson, Ph.D.
President and CEO

**DATE:** December 18, 2014

**RE:** Transfer of sole proprietary rights.

BioScience Laboratories, Inc., hereby grants to Global Environmental Restoration, Inc. permission to release the Final Report for Study #140821-404.01 including all test data and attendant documents, to the U.S. Environmental Protection Agency, or other government regulatory bodies, as appropriate. Implicit in this conveyance is relinquishment by BioScience Laboratories, Inc., of any and all proprietary rights to copyrighted forms and documents comprising the Final Report for the aforesaid study.

Daryl S. Paulson

12-18-14

Daryl S. Paulson, Ph.D.
President and CEO

Date