FINAL STUDY REPORT

Study Title
Virucidal Efficacy of a Test Substance for Use on Inanimate, Nonporous Surfaces

Test Substance
DoxyKlor DK5G
Lot Numbers: 06120-1/LCL, 06120-2/LCL

Test Microorganism
Adenovirus 5, Adenoid 75 strain, ATCC VR-5

Data Requirements
U.S. EPA OCSPP 810.2200

Author
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Study Director

Study Completion Date
24NOV2020

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

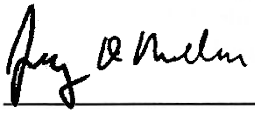
No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA section 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10(g).

Company: PI Industries, Inc.

Agent/Submitter: Jeremy D. Malone

Title: Consultant

Date: 11/24/2020

Signature: 

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR Part 160.

Records concerning test substance characteristics (i.e. composition, purity, stability, strength, solubility) are maintained by the Study Sponsor. The Study Sponsor conducted test substance characterization as to identity, strength, purity, solubility and composition, as applicable, according to 40 CFR Part 160, Subpart F [160.105] prior to its use in the study. The test substance certificate of analysis may be found attached to this report for reference.

Study Director

Company: Microchem Laboratory

Name: Victoria Zarate, B.S.

Title: Study Director

Signature: _____ Date: 24NOV2020

Study Sponsor

Company: PI Industries

Name: Jeremy Malone

Title: Study Sponsor

Signature: _____ Date: 11/24/2020

Submitter

Company: Spring Regulatory Sciences

Name: Jeremy D. Malone

Title: Consultant

Signature: _____ Date: 11/24/2020

QUALITY ASSURANCE STATEMENT

The following quality assurance audits were conducted in accordance with Good Laboratory Practice Standards outlined in 40 CFR §160 and reported to management and the Study Director:

Phase Inspected	Date Inspected	Date Reported to Study Director	Date Reported to Management
In Phase	02SEP2020	03SEP2020	03SEP2020
In Phase	28SEP2020	28SEP2020	28SEP2020
Draft Report	16NOV2020	16NOV2020	16NOV2020
Final Report	23NOV2020	23NOV2020	24NOV2020

Signature: _____ Date: 24 NOV 2020

Name: Alex Troxclair, B.S.
Title: Specialist I, Quality Assurance

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PERSONNEL INVOLVED IN THE STUDY

Study Director

Name: Victoria Zarate, B.S.
Title: Team Lead, Virology

Professional or Supervisory Personnel

Name: Emily Cox, B.S.
Title: Analyst I

Name: Tamisha Smith, B.S.
Title: Associate Analyst

Name: Aubrey Francione, B.S.
Title: Associate Analyst

TABLE OF CONTENTS

FINAL STUDY REPORT SUMMARY

Study Title: Virucidal Efficacy of a Test Substance for Use on Inanimate, Nonporous Surfaces

Study Identification Number: GLP2427

Test Microorganism: Adenovirus 5, Adenoid 75 strain, ATCC VR-5

Host Cell: A549, ATCC CCL-185

Test Substance: DoxyKlor DK5G
Lots: 06120-1/LCL, 06120-2/LCL

Test Substance Dilution: Ready-to-use liquid substance; no dilution required

Organic Soil Load: No additional soil load supplementation

Carrier Type: Sterile glass Petri dish (100 mm x 15 mm)

Number of Carriers Per Lot: 1

Contact Time(s): 10 minutes

Exposure Temperature: Ambient room temperature (23.9-24.3 °C and 29-30% Relative Humidity (RH))

Neutralization Method: Sephadex LH-20 gel filtration column

Study Results:

Assay Results			
Description	**Test Lot: 06120-1/LCL**	**Test Lot: 06120-2/LCL**	**Plate Recovery Control (Log_{10} $TCID_{50}$/Carrier)**
Log_{10} $TCID_{50}$/0.1 ml	≤0.50 log_{10}	≤0.50 log_{10}	6.30 log_{10}
Log_{10} $TCID_{50}$/Carrier	≤0.80 log_{10}	≤0.80 log_{10}	
Log_{10} Reduction/Carrier	≥5.50 log_{10}	≥5.50 log_{10}	

STUDY DATES

Study Initiation Date:	10AUG2020
Experimental Start Date/Time:	02SEP2020 / 1635
Experimental End Date/Time:	05OCT2020 / 1713
Study Completion Date:	24NOV2020

TEST SUBSTANCE

Name: DoxyKlor DK5G

Lot: 06120-1/LCL

Active Ingredients (concentration):	Chlorine dioxide (0.04385%)
Date of Manufacture:	01JUN2020
Date Received:	20AUG2020
Expiration Date:	01JUN2021

Lot: 06120-2/LCL

Active Ingredients (concentration):	Chlorine dioxide (0.04365%)
Date of Manufacture:	01JUN2020
Date Received:	20AUG2020
Expiration Date:	01JUN2021

Form: Ready-to-use liquid substance

Storage Conditions: Ambient room temperature under fluorescent lighting

Test Substance Preparation
The test substance was used as received, ready-to-use in a trigger spray bottle. The test substance appeared to be in solution as determined by visual observation on the day of use.

TESTING SYNOPSIS

This assay was originally conducted on 02SEP2020 and the results of the Plate Recovery Control were invalid as they did not meet the minimum recoverable virus endpoint of 4.8 log_{10} per carrier as required by the US EPA. All data from this test date are considered invalid. This assay was repeated on 28SEP2020 and valid Plate Recovery Control results were obtained. The valid data is presented in the body of this report and the invalid data is located in the Appendix.

PROTOCOL CHANGES

Protocol Amendment(s)

Protocol Amendment #1:

Per the Study Sponsor's request, in order to ensure timely study reporting and sufficient recovery of virus for the plate recovery control, the virus strain was changed from Adenovirus, Adenoid 71 strain, ATCC VR-1 to Adenovirus 5, Adenoid 75 strain, ATCC VR-5.

Protocol Amendment #2:

At the request of the Study Sponsor, an amendment was made to protocol P2847 to include an expiration date for the provided test substance. On 26OCT2020, the Study Sponsor presented a revised version of the certificate of analysis to replace that of which was previously provided for protocol P2847. The certificate of analysis was updated to include the expiration date for test substance DoxyKlor DK5G (Lots: 06120-1/LCL and 06120-2/LCL) as 01JUN2021, one year from the date of manufacture.

Protocol P2847 is hereby amended to include the expiration date of the test substance, as requested by the Study Sponsor.

All remaining testing parameters are to be followed as stated in the protocol.

Protocol Deviation(s)

There were no deviations from the approved protocol during the conduct of this study.

TEST OBJECTIVE

The purpose of this study is to document the virucidal efficacy of the test substance against the test system (microorganism) under the test parameters specified in the protocol.

TEST PROCEDURE

Test System (Microorganism)

Adenovirus 5, Adenoid 75 strain, ATCC VR-5, originally received from the American Type Culture Collection (ATCC), Manassas, VA, was used in this study. The Microchem Laboratory lot number used in testing was ADV5_26AUG2020A.

Preparation of the Test Virus

The test virus was propagated internally by Microchem Laboratory personnel by inoculating the virus into cell culture flasks containing the appropriate host cell line and incubating at the appropriate conditions. Once the cell culture flasks displayed approximately 75-100% cytopathic effect (as determined by microscopic evaluation), the flasks were subjected to freeze-thaw cycles to release virus from infected cells. The contents of the cell culture flasks were collected and centrifuged in order to remove the cell debris. The test virus was then aliquoted and stored at ≤-70 °C.

On the day of testing, an aliquot of the virus stock suspension was removed from cryostorage and thawed for use in the assay. The test virus contained 2% fetal bovine serum (FBS) organic soil load. The test virus was not adjusted to incorporate any additional organic soil load into the inoculum.

Host Cell-Line

A549 cells, (ATCC CCL-185) originally received from ATCC were utilized in the assay. The cells were subcultured by Microchem Laboratory personnel and seeded into 24 well cell culture plates. The plates were incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 until they reached the desired confluence required for testing. On the day of use, the cells were microscopically examined to verify the appropriate confluency and health of the cells.

Test Medium

The test medium utilized in the assay was Eagle's Minimum Essential Medium (EMEM) supplemented with 2% FBS plus antibiotics [100 µg/ml kanamycin sulfate solution and antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)].

Preparation of the Test Substance

The test substance was used as directed by the Study Sponsor.

The test substance was used as received, ready-to-use in a trigger spray bottle. The test substance appeared to be in solution as determined by visual observation on the day of use.

TEST PROCEDURE (cont.)

Preparation of Sephadex LH-20 Gel Filtration Columns

Sephadex LH-20 gel filtration columns were utilized to neutralize and/or to reduce the cytotoxicity of the test substance following exposure to the test virus. On the day of testing, the prepared Sephadex slurry was aseptically added to prepared column units (sterile syringe) to completely fill the column. Just prior to testing, the syringe was centrifuged at approximately 100 x *g* for 3-4 minutes to clear the void volume.

Preparation of Virus Films

The test virus was vortexed thoroughly and a 0.2 ml aliquot was placed on the inside bottom surface of three 100 x 15 mm sterile glass Petri dishes which served as the test carriers and plate recovery control. The inoculum was then spread over the entire area of the carriers using a sterile bent pipette tip without touching the sides of the Petri dish. The virus films were dried in an environmental chamber for 21 minutes at 20.0 °C in a relative humidity of 30%.

Exposure of Virus Films to the Test Substance

For each lot of the test substance, one dried virus film carrier was treated with the amount of spray released following the Study Sponsor's instructions. The carriers were sprayed with 5 sprays at a distance of 4-6 inches from the carrier surface. The carriers were gently rotated to ensure complete coverage of the test substance over the entirety of each test surface. The carriers were held covered at an exposure temperature of 23.9-24.3 °C in a relative humidity of 29-30% for the requested contact time of 10 minutes. Just prior to the completion of the contact time, sterile cell scrapers were used to re-suspend the viral films and the solution was immediately transferred into individual gel filtration columns. The syringe plunger was used to pass the contents of the re-suspended test carrier through the column. Serial 10-fold dilutions of the filtrate were prepared using test media by adding 0.1 ml filtrate to 0.9 ml test media.

STUDY CONTROLS

Plate Recovery Control

One plate recovery control film was prepared to determine the baseline dried virus titer. The plate recovery control film was generated as described above in "Preparation of Virus Films." Following drying, a 2.0 ml aliquot of test medium was overlaid on the control film. The carrier was then gently rotated to ensure complete coverage of the solution over the entirety of the surface. The carrier was held covered at an exposure temperature of 24.2 °C in a relative humidity of 29-30% for 10 minutes. Just prior to the completion of the study contact time, a sterile cell scraper was used to re-suspend the viral film and the solution was immediately transferred into a gel filtration column. The re-suspended contents of the carrier were passed through the gel filtration column using the syringe plunger. Serial 10-fold dilutions of the filtrate were prepared using test media by adding 0.1 ml filtrate to 0.9 ml test media.

Cytotoxicity Control

For each lot of test substance assayed, one sterile glass Petri dish carrier (containing no virus film) was treated in the same manner as the test carriers. The carriers were treated with the amount of spray released following the Study Sponsor's instructions. The carriers were sprayed with 5 sprays at a distance of 4-6 inches from the carrier surface. The carriers were gently rotated to ensure complete coverage of the test substance over the entirety of each test surface. The carriers were held covered at an exposure temperature of 23.7-24.3 °C in a relative humidity of 30-32% for the requested contact time of 10 minutes. Just prior to the completion of the study contact time, the carriers were scraped using sterile cell scrapers and the test substance suspensions were promptly transferred into individual gel filtration columns. The re-suspended test substances were passed through the gel filtration columns using the syringe plunger. Serial 10-fold dilutions of the filtrate were prepared using test media by adding 0.1 ml filtrate to 0.9 ml test media.

Test Substance Neutralization Control

For each lot of test substance assayed, one sterile glass Petri dish carrier (containing no virus film) was treated in the same manner as the test carriers. The carriers were treated with the amount of spray released following the Study Sponsor's instructions. The carriers were sprayed with 5 sprays at a distance of 4-6 inches from the carrier surface. The carriers were gently rotated to ensure complete coverage of the solution over the entirety of each test surface. The carriers were scraped using sterile cell scrapers and the test substance suspensions were promptly transferred into individual gel filtration columns. The re-suspended test substances were passed through the gel filtration columns using the syringe plunger. A 2.0 ml aliquot of test media was passed through a gel filtration column in the same manner as the test to serve as a neutralization control substance.

To verify that the test substance had been neutralized, the filtrate (neutralized test substance) and the neutralization control substance were each challenged with a 0.1 ml aliquot of low titer (e.g. 1000-5000) infective units of the test system and held for 10 minutes at an exposure temperature of 23.8-24.3 °C in a relative humidity of 29-30%. Serial 10-fold dilutions of the filtrate were prepared using test media by adding 0.1 ml filtrate to 0.9 ml test media.

STUDY CONTROLS (cont.)

Cell Culture Control

To ensure that the host cells were not contaminated with bacteria, fungi, or any cytopathogenic viruses, and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers were left untreated and microscopically examined periodically throughout the incubation period. Any obvious contamination or degeneration in such monolayers could invalidate the virucidal efficacy assay.

Virus Inoculum Titer Control

To confirm that the host cell-line monolayers were susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the test virus inoculum was serially diluted 10-fold in test media.

Infectivity Assay

A 0.1 ml aliquot of all test and control dilutions were inoculated into the host cells cultures (which contain test medium) in quadruplicate. The cell culture controls wells contained just test medium. The assay plates were incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 for 7 days. The assay plates were examined microscopically periodically throughout the incubation period with any changes to the monolayers including viral cytopathic effects (CPE), cytotoxicity, or contamination clearly documented in the raw data. Data obtained from the final reading are documented in the Results section of this report.

SUCCESS CRITERIA

The following measures are met to ensure the acceptability of virucidal efficacy data:

- The virus titer control demonstrate obvious and/or typical cytopathic effects on the monolayers unless a detection method other than cytopathic is used.
- A minimum of 4.80 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film(s).
- If cytotoxicity is present, the virus control titer is sufficient to demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxic level.
- Comparable levels of infective units must be recovered from the neutralized test substance and neutralization control substance.
- Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.
- The cell controls are negative for infectivity and demonstrate typical cell morphology.

The U.S. EPA performance criteria for disinfection follows:

- In the presence or absence of cytotoxicity, the product should demonstrate a $\geq$3.00 $\log_{10}$ reduction in viral titer on each surface.

Retesting Guidance

- When a test passes and the $TCID_{50}$ of the plate recovery control is above 6.3 $\log_{10}$ infective units per carrier, no retesting is necessary.
- When a test fails and the $TCID_{50}$ of the plate recovery control is above 6.3 $\log_{10}$ infective units per carrier, retesting may be repeated.
- When a test fails and the $TCID_{50}$ of the plate recovery control is below 4.80 $\log_{10}$ infective units per carrier, no retesting is necessary.
- When cytotoxicity is present and a $\geq$3.00 $\log_{10}$ reduction is observed, no retesting is necessary.
- When cytotoxicity is present and a $\leq$3.00 $\log_{10}$ reduction is observed, retesting may be necessary.

CALCULATIONS AND STATISTICAL ANALYSIS

The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$ and TCD_{50} were determined using the Spearman-Kärber method and calculated as follows:

Negative logarithm of endpoint titer =

[– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

The result of this calculation is expressed as $TCID_{50}$/volume of dilution inoculated (e.g. 0.1 ml) for the test and plate recovery control. Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier.

To calculate $TCID_{50}$/carrier, the following equation was used:

The antilog of the $TCID_{50}$/volume inoculated x (volume of inoculum on carrier/volume of dilution inoculated)

The log_{10} of this result is performed to achieve the $TCID_{50}$/carrier.

Calculation of the Log_{10} Reduction

The log_{10} reduction in viral titer was calculated as follows:

Plate Recovery Control Log_{10} $TCID_{50}$/carrier – Virus-Test Substance Log_{10} $TCID_{50}$/carrier

The presence of any test substance cytotoxicity was taken into account when calculating the log_{10} reduction in viral titer.

If multiple plate recovery control and test replicates were performed, the average $TCID_{50}$ of each parameter was calculated and the average result used to calculate the log_{10} reduction in viral titer.

Statistical Analysis
Not applicable.

Methods for the Control of Bias
Not applicable.

DATA AND SAMPLE RETENTION

Study Record Retention

The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely. For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsor's archive at their expense.

If requested by the Study Sponsor (or Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.

All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.

Test Substance Retention

The test substance (or test control, test article, test device, as applicable) may be returned to the Study Sponsor at the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

RESULTS

Table 1: Plate Recovery Control and Test Results

		Plate Recovery Control	Test Results DoxyKlor DK5G Lot: 06120-1/LCL	Test Results DoxyKlor DK5G Lot: 06120-2/LCL
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	0 0 0 0	0 0 0 0
	10^{-2}	+ + + +	0 0 0 0	0 0 0 0
	10^{-3}	+ + + +	0 0 0 0	0 0 0 0
	10^{-4}	+ + + +	0 0 0 0	0 0 0 0
	10^{-5}	+ + + +	0 0 0 0	0 0 0 0
	10^{-6}	0 + 0 +	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		6.00 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}
$TCID_{50}$ per Carrier		6.30 log_{10}	≤0.80 log_{10}	≤0.80 log_{10}
Log_{10} Reduction per Carrier		N/A	≥5.50 log_{10}	≥5.50 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

Table 2: Cytotoxicity Control Results

		Cytotoxicity Control DoxyKlor DK5G Lot: 06120-1/LCL	Cytotoxicity Control DoxyKlor DK5G Lot: 06120-2/LCL
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0
TCD_{50} per 0.1 ml		≤0.50 log_{10}	≤0.50 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

RESULTS (cont.)

Table 3: Test Substance Neutralization Control Results

		Neutralization Control DoxyKlor DK5G Lot: 06120-1/LCL	Neutralization Control DoxyKlor DK5G Lot: 06120-2/LCL	Neutralization Control: Test Media
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ + + +	+ + + +	+ + + +
	10^{-3}	+ + + +	+ + + +	+ + + +
	10^{-4}	+ + 0 0	0 + 0 +	+ + 0 +
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		4.00 log_{10}	4.00 log_{10}	4.25 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

Table 4: Virus Inoculum Titer Control

		Virus Inoculum Titer Control
Cell Control		0 0 0 0
Dilution	10^{-1}	+ + + +
	10^{-2}	+ + + +
	10^{-3}	+ + + +
	10^{-4}	+ + + +
	10^{-5}	+ + + +
	10^{-6}	+ + + +
	10^{-7}	0 0 0 0
	10^{-8}	0 0 0 0
$TCID_{50}$ per 0.1 ml		6.50 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

STUDY CONCLUSION

The purpose of the study was to determine the virucidal efficacy of DoxyKlor DK5G (Lots: 06120-1/LCL and 06120-2/LCL) against Adenovirus 5, Adenoid 75 strain, ATCC VR-5 with no additional soil load incorporated into the inoculum, at a contact time of 10 minutes and an exposure temperature of room temperature.

The Plate Recovery Control demonstrated a viral titer of 6.00 $\log_{10}$ $TCID_{50}$ per 0.1 ml and 6.30 $\log_{10}$ $TCID_{50}$ per carrier, thereby satisfying U.S. EPA study acceptance criteria of a minimum of 4.80 $\log_{10}$ infective units per control carrier.

Taking the cytotoxicity and neutralization control results into consideration, the evaluated test substance demonstrated a $\geq$5.50 $\log_{10}$ reduction in viral titer for both lots assayed.

No test substance cytotoxic effects to the host monolayer were observed in any dilution assayed for either lot of test substance ($\leq$0.80 $\log_{10}$ TCD_{50} per 0.1 ml).

The test substance and control substance demonstrated comparable levels of infective units recovered in the Neutralization Control.

No microbial contamination of any host cell cultures was observed during the course of the study.

DoxyKlor DK5G (Lots: 06120-1/LCL and 06120-2/LCL) met the U.S. EPA Product Performance Guidelines for Disinfectants for Use on Hard Surfaces outlined in U.S. EPA OCSPP 810.2200 and the success criteria detailed in the approved protocol when tested against Adenovirus 5, Adenoid 75 strain, ATCC VR-5 at a contact time of 10 minutes.

This study was carried out in compliance with the approved protocol. All experimental controls met the established acceptance criteria unless otherwise noted in the Testing Synopsis and/or Protocol Changes sections of this report.

There were no circumstances that may have affected the quality of the integrity of the data.

REFERENCES

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

- *Annual Book of ASTM Standards*, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

- *Official Methods of Analysis of the AOAC International,* Chapter 6, Disinfectants, Official Method 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants. Revised 2013.

- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.

- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.

- U.S. Environmental Protection Agency, Frequent Questions for the 2018 series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. 2019.

- Guidance Document – Disinfectant Drugs. Health Canada. January 2018.

- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. January 2014.

APPENDIX

Table A-1: Plate Recovery Control and Test Results for Testing Performed on 02SEP2020

		Plate Recovery Control	Test Results DoxyKlor DK5G Lot: 06120-1/LCL	Test Results DoxyKlor DK5G Lot: 06120-2/LCL
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	0 0 0 0	0 0 0 0
	10^{-2}	+ + + +	0 0 0 0	0 0 0 0
	10^{-3}	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
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-7}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		2.50 log_{10}	≤0.50 log_{10}	≤0.50 log_{10}
$TCID_{50}$ per Carrier		2.80 log_{10}	≤0.80 log_{10}	≤0.80 log_{10}
Log_{10} Reduction per Carrier		N/A	≥2.00 log_{10}	≥2.00 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

Table A-2: Cytotoxicity Control Results for Testing Performed on 02SEP2020

		Cytotoxicity Control DoxyKlor DK5G Lot: 06120-1/LCL	Cytotoxicity Control DoxyKlor DK5G Lot: 06120-2/LCL
Cell Control		0 0 0 0	0 0 0 0
Dilution	10^{-1}	0 0 0 0	0 0 0 0
	10^{-2}	0 0 0 0	0 0 0 0
	10^{-3}	0 0 0 0	0 0 0 0
TCD_{50} per 0.1 ml		≤0.50 log_{10}	≤0.50 log_{10}

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed;
T = Cytotoxicity observed

APPENDIX (cont.)

Table A-3: Test Substance Neutralization Control Results for Testing Performed on 02SEP2020

		Neutralization Control DoxyKlor DK5G Lot: 06120-1/LCL	Neutralization Control DoxyKlor DK5G Lot: 06120-2/LCL	Neutralization Control: Test Media
Cell Control		0 0 0 0	0 0 0 0	0 0 0 0
Dilution	10^{-1}	+ + + +	+ + + +	+ + + +
	10^{-2}	+ 0 0 0	0 0 0 0	0 0 0 0
	10^{-3}	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
	10^{-5}	0 0 0 0	0 0 0 0	0 0 0 0
	10^{-6}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$ per 0.1 ml		1.75 $\log_{10}$	1.50 $\log_{10}$	1.50 $\log_{10}$

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

Table A-4: Virus Inoculum Titer Control for Testing Performed on 02SEP2020

		Virus Inoculum Titer Control
Cell Control		0 0 0 0
Dilution	10^{-1}	+ + + +
	10^{-2}	+ + + +
	10^{-3}	+ + + +
	10^{-4}	0 0 0 0
	10^{-5}	0 0 0 0
	10^{-6}	0 0 0 0
	10^{-7}	0 0 0 0
	10^{-8}	0 0 0 0
$TCID_{50}$ per 0.1 ml		3.50 $\log_{10}$

Key: + = Virus recovered; 0 = Virus not recovered and/or no cytotoxicity observed; T = Cytotoxicity observed

PROTOCOL AMENDMENT (S)

Protocol Amendment for Protocol P2847, Study ID Number GLP2427

Protocol Amendment #2

Protocol P2847 is hereby amended to include the expiration date of the test substance per clarification from the Sponsor Representative.

Expiration date for DoxyKlor DK5G is 1 year from manufacture. Expiration date for Lots: 06120-1/LCL and 06120-2/LCL is 01JUN2021.

All remaining testing parameters are to be followed as stated in the protocol.

Study Director (signature) — 18 NOV 2016 Date Signed

Victoria Zarate
Study Director (print)

PROTOCOL AMENDMENT(S) (cont.)

MICROCHEM LABORATORY

Protocol Amendment for Protocol P2847, Study ID Number GLP2427

Protocol Amendment #1

Per the Study Sponsor's request, in order to ensure timely study reporting and sufficient recovery of virus for the plate recovery control, the virus strain was changed from Adenovirus, Adenoid 71 strain, ATCC VR-1 to Adenovirus 5, Adenoid 75 strain, ATCC VR-5.

All remaining testing parameters are to be followed as stated in the protocol.

Study Sponsor (signature) — 9/24/2020 Date Signed

Jeremy D. Malone
Study Sponsor (print)

Study Director (signature) — 24SEP2020 Date Signed

Victoria Zavala
Study Director (print)

PROTOCOL

MICROCHEM LABORATORY

Protocol Number: P2847 Study ID Number: GLP 2427

Protocol Title
Virucidal Efficacy of a Test Substance for Use on Inanimate, Nonporous Surfaces

Test Microorganism
Adenovirus, Adenoid 71 Strain, ATCC VR-1

Data Requirements
U.S. EPA OCSPP 810.2200

Sponsor Representative
Jeremy Malone
Spring Regulatory Sciences
6620 Cypresswood Dr. Ste 250
Spring, TX 77379

Study Sponsor
PI Industries
8275 S. Eastern Avenue Suite#200-882
Las Vegas, NV 89123-2545

Testing Facility
Microchem Laboratory
1304 W. Industrial Blvd.
Round Rock, Texas 78681

Prepared By:
Victoria Zarate, B.S.

Date
07JUL2020

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the virucidal efficacy of a test substance for use on inanimate, nonporous surfaces following test method ASTM E1053 Standard Practice to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces. Testing will be performed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by U.S. EPA 40 CFR Part 160 as well as the U.S. EPA Product Performance Test Guidelines outlined in OCSPP 810.2200. This document also explains the terms and conditions of testing.

II. Purpose

The purpose of this study is to document the virucidal efficacy of the test substance against the test system (microorganism) under the test parameters specified in this protocol.

III. Justification for the Selection of the Test System (Microorganism)

The United States Environmental Protection Agency (U.S. EPA) requires that specific antimicrobial claims made for disinfectants for use on hard surfaces sold in the United States be supported by relevant test systems (microorganisms) outlined in the EPA Product Performance Test Guidelines, OCSPP 810.2200, Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing.

IV. Terms and Conditions

Studies by Microchem Laboratory are conducted in accordance with general terms and conditions posted on www.MicrochemLab.com/terms.

Prior to study initiation, Microchem Laboratory should receive the approved and signed protocol, test substance and payment. Changes to the signed, approved protocol will require amendment and may incur additional fees. Cancellation of the study any time after study initiation may result in a cancellation fee of up to 100% of the total study cost, to be determined by laboratory management at its sole discretion.

Microchem Laboratory may repeat studies at its cost in the event of an unintended protocol non-conformance that affects the study outcome, or for studies which yield invalid control results. If the Sponsor requests a specific neutralizer to be utilized in testing and test controls indicate incomplete or inadequate neutralization, repeat testing will be at the Study Sponsor's expense for applicable testing. Repeat testing may be conducted under the current initiated protocol and Microchem Laboratory GLP study identification number. In addition, the Study Sponsor is responsible for the cost of all studies performed to confirm the outcome of a previous study and for ensuring that the study will meet their regulatory objectives.

The Study Sponsor must obtain written consent from Microchem Laboratory to use or publish its protocols, study reports (or parts thereof), logo or employee names for marketing purposes.

PROTOCOL (cont.)

V. Test Substance Characterization and Handling

As stated in 40 CFR Part 160 Subpart F [160.105], each batch (lot) of test substance shall be characterized as to identity, strength, purity, composition, and solubility (as applicable), and shall be documented prior to use in this assay. Stability of the test formula shall be determined prior to or concomitantly with this study. If the requirements set forth in 40 CFR Part 160 Subpart F [160.105] have not been met, this will be noted in the Good Laboratory Practice compliance statement in the study report. Certificates of Analysis (C of A) will be appended to the study report, if provided by the Study Sponsor.

Test substances are handled as follows unless otherwise requested by the Study Sponsor:

- The test substance is stored at ambient (room) temperature under fluorescent lighting or in a cabinet.
- The test substance is shaken or otherwise mixed well immediately prior to use (if applicable).
- The test substance is handled safely in accordance with the chemical risks it may pose, stated in the SDS or by the Study Sponsor during the course of pre-study communication.

VI. Study Dates

The listed proposed experimental start and completion dates are estimates based on the current laboratory schedule and may change based on when the test substance, sponsor signed protocol, and payment (if applicable) are received at the testing laboratory. To avoid scheduling delays, assure that all paperwork is completed fully and accurately.

Proposed Experimental Start Date: **24JUL2020**
Proposed Experimental Termination Date: **31JUL2020**

VII. Procedure for Identification of Test System

Microchem Laboratory maintains Standard Operating Procedures which outline the procedures for receipt, storage, and tracking of microorganisms. The vessels, racks, and trays containing the test system are labeled with microorganism identifiers to maintain microorganism traceability. Information regarding the microorganism identity, strain, propagation procedure, media utilized, etc. is documented in the study raw data. All studies are assigned a unique identification number which is labeled on the test and control vessels, racks, trays, etc. These procedures are followed to identify and document the test system.

VIII. Test System (Microorganism)

Adenovirus, Adenoid Strain 1, ATCC VR-1

The virus to be used in this study was originally obtained from the American Type Culture Collection (ATCC), Manassas, Virginia. The source of the virus will be documented in the raw data and report.

PROTOCOL (cont.)

IX. Procedure

Preparation of the Test Virus

- The test virus is propagated internally by Microchem Laboratory personnel by inoculating the virus into cell culture flasks containing the appropriate host cell line and incubating at the appropriate conditions.
- Once the cell culture flask(s) display approximately 75-100% cytopathic effect (as determined by microscopic evaluation), the flasks are subjected to freeze thaw cycles to release virus from infected cells.
- The contents of the cell culture flask(s) are collected and centrifuged in order to remove the cell debris.
- The test virus is then aliquoted and stored at ≤-70 °C.
- Alternate methods of propagation and harvesting may be utilized as necessary for the test virus. The propagation procedure is documented and will be reported.
- On the day of testing, the appropriate number of virus stock suspension vials are removed from cryostorage and thawed. The test virus may be standardized by dilution as needed to target a recoverable plate recovery control of 4.8 $\log_{10}$ to 6.3 $\log_{10}$ infective units per plate recovery control or 3-5 $\log_{10}$ beyond the level of cytotoxicity.
- If the Study Sponsor requests an organic soil to be incorporated into the test virus, it will be added following any standardization of the test virus.

Host Cell-Line

- A549 cells (ATCC CCL-185) originally received from ATCC will be utilized in the assay. If necessary, cells received from an alternate source may be utilized. The original source of the cells will be documented in the raw data and reported.
- The cells will be subcultured by Microchem Laboratory personnel and seeded into 24-well cell culture plates.
- The plates are incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 until they have reached the desired confluence required for testing.

Test Medium

- The test medium to be utilized in the assay is Eagle's Minimum Essential Medium (EMEM) or Dulbecco's Modified Eagle Medium (DMEM) which has been supplemented with 0-10% fetal bovine serum (FBS). The test medium may also contain additional supplements such as antibiotics, fungizone, L-glutamine, trypsin, non-essential amino acids, etc., depending on the requirements of the test virus and/or host cells. The final composition of the test media utilized in the assay will be documented in the raw data and reported.

Preparation of the Test Substance

- The test substance will be used as directed by the Study Sponsor.
- Unless otherwise requested by the Study Sponsor, if a dilution of the test substance is required, a ≥1.0 ml or ≥1.0 g of the test substance will be used to prepare the test substance using volumetric glassware. For liquid products, a v/v dilution will be made and for solids, a w/v dilution will be made.
- If synthetic hard water is requested as the diluent, it will be prepared following Microchem Laboratory Standard Operating Procedures for the specific water type. The hardness range will be -10% to +5% of the specified hardness.
- If tap water is requested as the diluent, the water will be autoclave sterilized. The water hardness will be determined on the day of testing and adjusted to the hardness range if necessary.
- The test substance will be equilibrated to the requested exposure temperature, if applicable, prior to use.

PROTOCOL (cont.)

Preparation of Sephadex LH-20 Gel Filtration Columns

- Sephadex LH-20 gel filtration columns will be utilized to neutralize and/or to reduce the cytotoxicity of the test substance following exposure to the test virus.
- On the day of testing, the prepared Sephadex slurry is aseptically added to prepared column units (sterile syringe) to completely fill the column.
- Just prior to testing, the syringe is centrifuged at approximately 100 x g for 3-4 minutes to clear the void volume.
- Alternatively, Sephacryl may be utilized in place of Sephadex, in which case, preparation will be conducted according to internal SOP, documented, and reported.

Preparation of Virus Films

- The test virus is vortexed thoroughly and a 0.2 ml aliquot is placed on the inside bottom surface of the appropriate number of 100 x 15 mm sterile glass Petri dishes which serve as the test carriers.
  - A larger inoculum volume may be used as necessary in order to ensure an appropriate viral inoculum titer. The volume of test virus utilized will be documented in the raw data and reported.
- The inoculum is then spread over the entire area of the carriers using a sterile cell scraper tool or bent pipette tip without touching the sides of the Petri dish.
- The virus films are dried in a biosafety cabinet or other suitable chamber at the temperature and humidity conditions appropriate to lessen the levels of virus inactivation due to drying with the Petri dish covers removed. The viral inoculum is allowed to dry until the surface appears to be visibly dry. The temperature, relative humidity, and drying time period will be recorded in the raw data and reported.

Exposure of Virus Films to the Test Substance

- For each lot of the test substance, the appropriate number of dried virus film carriers are treated with a 2.0 ml aliquot of the use dilution of the liquid test substance or for spray products, with the amount of spray released following the Study Sponsor instructions. The carriers are gently rotated to ensure complete coverage of the test substance over the entirety of each test surface.
- The carriers are held covered at the Study Sponsor requested exposure temperature for the requested contact time. The exposure conditions (contact time, temperature, and relative humidity) will be documented in the raw data and reported.
- Just prior to the completion of the contact time, sterile cell scrapers are used to re-suspend the viral films and the solution is immediately transferred into gel filtration columns.
- The syringe plunger is used to pass the contents of the re-suspended test carrier through the column. Alternatively, the re-suspended contents may be passed through the gel filtration column by centrifugation at 100 x *g* for 3-4 minutes.
- Serial 10-fold dilutions of the filtrate (e.g. 0.1 ml filtrate + 0.9 ml test media) are prepared to the appropriate dilution.
- If excessive cytotoxicity of the test substance to the indicator cell cultures is suspected, following titration, individual dilutions may be passed through additional individual gel filtration columns.

Plate Recovery Control

- An appropriate number of plate recovery control films will be prepared to determine the baseline dried virus titer. The plate recovery control films will be generated as described above in "Preparation of Virus Films."
- Following drying, a 2.0 ml aliquot of test medium is overlayed on each control film.
- The carrier is then gently rotated to ensure complete coverage of the solution over the entirety of the surface. All other test conditions and parameters (e.g. Sponsor requested contact time and exposure temperature) will be the same as the test carriers.
- Just prior to the completion of the study contact time, sterile cell scrapers are used to re-suspend the viral films and the solution is immediately transferred into gel filtration columns.
- The re-suspended contents of each carrier is passed through the gel filtration column in the same manner as the test carriers.

PROTOCOL (cont.)

Plate Recovery Control (Continued)

- Serial 10-fold dilutions (e.g. 0.1 ml filtrate + 0.9 ml test media) of the filtrate are prepared to the appropriate dilution.
- If additional gel filtration columns were utilized in the test, the same dilutions of the plate recovery control will be passed through individual gel filtration columns.

Cytotoxicity Control

- For each lot of test substance assayed, one sterile glass Petri dish carrier (containing no virus film) is treated in the same manner as the test carriers. A 2.0 ml aliquot of the use dilution of the test substance for liquid products or for spray products, the amount of test substance delivered following the Sponsor specified spray instructions is added to the sterile Petri dish and held for the Study Sponsor requested study contact time at the requested exposure temperature.
- Just prior to the completion of the study contact time, the carrier is scraped using a sterile cell scraper and the test substance suspension is promptly transferred into a gel filtration column.
- The re-suspended test substance is passed through the gel filtration column in the same manner as the test.
- Serial 10-fold dilutions (e.g. 0.1 ml filtrate + 0.9 ml test media) are prepared to the appropriate dilution.

Test Substance Neutralization Control

- For each lot of test substance assayed, one sterile glass Petri dish carrier (containing no virus film) is treated in the same manner as the test carriers. A 2.0 ml aliquot of the use dilution of the test substance for liquid products or for spray products, the amount of test substance delivered following the Sponsor specified spray instructions is added to the sterile Petri dish.
- The carrier is scraped using a sterile cell scraper and the test substance suspension is promptly transferred into a gel filtration column.
- The re-suspended test substance is passed through the gel filtration column in the same manner as the test.
- A 2.0 ml aliquot of test media, or other media as appropriate, is passed through the gel filtration column in the same manner as the test to serve as a neutralization control substance to determine if comparable levels of infectious viral units are recovered from the control and the neutralized test substance filtrate.
- To verify that the test substance has been neutralized, the filtrate (neutralized test substance) and the neutralization control substance are each challenged with a 0.1 ml aliquot of low titer (e.g. 1000 – 5000) infective units of the test system and held for at least the contact time at room temperature. Serial 10-fold dilutions (e.g. 0.1 ml filtrate + 0.9 ml test media) are prepared using test media, to the appropriate dilution.

Cell Culture Control

- To ensure that the host cells are not contaminated with bacteria, fungi, or any cytopathogenic viruses and to confirm the viability of the cells during the incubation period of the assay, at least four host cell monolayers are left untreated, and will be microscopically examined periodically throughout the incubation period. Any obvious contamination or degeneration in such monolayers may invalidate the virucidal efficacy assay.

Virus Inoculum Titer Control

- To confirm that the host cell-line monolayers are susceptible to the test virus and to confirm the titer of the viral inoculum, an aliquot of the test virus inoculum is serially diluted (10-fold) in test media. This control is also used to confirm the level of virus inoculated in the Neutralization Control.

PROTOCOL (cont.)

Infectivity Assay

- A 0.1 ml aliquot of all test and control dilutions will be inoculated into the host cells cultures (which contain test medium) in quadruplicate.
- To facilitate virus-host cell adsorption, an adsorption step may be performed by inoculating the dilutions into the host cell cultures which do not contain test medium. The assay plates are incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 for a minimum of 30 minutes. The plates may also be placed upon an orbital rotator during this incubation period.
  - Following the optional adsorption, each well receives an approximate 1.0 ml aliquot of test medium via pipette delivery.
- The assay plates are incubated at 36 ± 2 °C in a humidified atmosphere of 6 ± 1% CO_2 for approximately 7 days.
- If necessary, test medium may be replaced during the incubation period to aid in reducing cytotoxicity and/or to maintain the health of the host cell cultures.
- The assay plates will be examined microscopically periodically throughout the incubation period with any changes to the monolayers including viral cytopathic effects (CPE), cytotoxicity, or contamination clearly documented in the raw data.

X. Calculations

- The $TCID_{50}$ (Tissue Culture Infectivity Dose) represents the endpoint dilution where 50% of the cell cultures exhibit cytopathic effects due to infection by the test virus. The endpoint dilution at which 50% of the host cell monolayers exhibit cytotoxicity is termed the Tissue Culture Dose (TCD_{50}). The $TCID_{50}$, and TCD_{50} are determined using the Spearman-Kärber method and calculated as follows:

  Negative logarithm of endpoint titer =

  [– Log of first dilution inoculated] – [((sum of % mortality at each dilution/100) – 0.5) x Logarithm of dilution]

  The result of this calculation is expressed as $TCID_{50}$/volume of dilution inoculated (e.g. 0.1 ml) for the test and plate recovery control.

- Determination of viral titer per carrier is established by accounting for the volume of viral inoculum per carrier.

  To calculate the $TCID_{50}$/carrier the following equation is used:

  The antilog of the $TCID_{50}$/volume inoculated x (volume of inoculum on carrier/volume of dilution inoculated)

  The log_{10} of this result is performed to achieve the $TCID_{50}$/carrier

Calculation of the Log Reduction

- The log reduction in viral titer will be calculated as follows:

  Plate Recovery Control Log_{10} $TCID_{50}$/carrier – Virus-Test Substance Log_{10} $TCID_{50}$/carrier

- The presence of any test substance cytotoxicity will be taken into account when calculating the log reduction in viral titer.
- If multiple plate recovery control and test replicates are performed, the average $TCID_{50}$ of each parameter will be calculated and the average result used to calculate the log reduction in viral titer.

PROTOCOL (cont.)

XI. Statistical Analysis

Not applicable.

XII. Methods for the Control of Bias

Not applicable.

XIII. Success Criteria

- The following measures are met to ensure the acceptability of virucidal efficacy data:
  - The virus titer control demonstrate obvious and or typical cytopathic effects on the monolayers unless a detection method other than cytopathic effect is used.
  - A minimum of 4.80 $\log_{10}$ infective units/control carrier is recovered from each plate recovery control film(s).
  - If cytotoxicity is present, the virus control titer is sufficient to demonstrate a ≥3.00 $\log_{10}$ reduction in viral titer on each surface beyond the cytotoxic level.
  - Comparable levels of infective units must be recovered from the neutralized test substance and neutralization control substance.
  - Quantification of the test and control parameters are conducted at a minimum of four determinations per dilution.
  - The cell controls are negative for infectivity and demonstrate typical cell morphology.

XIV. Product Performance Criteria

- The U.S. EPA performance criteria for disinfection follows:
  - In the presence or absence of cytotoxicity, the product should demonstrate a ≥3.00-$\log_{10}$ reduction in viral titer on each surface.

- Retesting Guidance
  - When a test passes and the $TCID_{50}$ of the plate recovery control is above 6.3 $\log_{10}$ infective units/per carrier, no retesting is necessary.
  - When a test fails and the $TCID_{50}$ of the plate recovery control is above 6.3 $\log_{10}$ infective units/per carrier, retesting may be repeated.
  - When a test fails and $TCID_{50}$ of the plate recovery control is below 4.80 $\log_{10}$ infective units/per carrier, no retesting is necessary.
  - When cytotoxicity is present and a ≥3.00 log reduction is observed, no retesting is necessary.
  - When cytotoxicity is present and a ≤3.00 log reduction is observed, retesting may be necessary.

XV. Reporting

- Results are reported accurately and fully, in accordance with EPA GLP (40 CFR Part 160). A draft report may be provided for review by the Study Sponsor prior to study completion.

PROTOCOL (cont.)

XVI. Study Record and Test Substance Retention

- The original (or certified copy) of the study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory indefinitely. For studies not meeting the performance criteria for submission or for studies that have been canceled prior to the generation of valid data, the original (or certified copy) of the final study report, protocol, and corresponding raw data will be held in the archives of Microchem Laboratory for a minimum of two years following the study completion date at which time they may be removed from the archive or transferred to the Sponsors archive at their expense.
- If requested by the Study Sponsor (or Sponsor Representative), the study file may be transferred to the Study Sponsor's archive at the Study Sponsor's expense prior to the time frames listed.
- All test facility records including, but not limited to, standard operating procedures, quality assurance inspection records, temperature and equipment records including maintenance, inspection and calibration, and employee training records will be maintained at Microchem Laboratory indefinitely.
- The test substance (or test control, test article, test device, as applicable) may be returned to the Study Sponsor at the Study Sponsor's request and expense following study completion unless otherwise requested to be returned earlier. If the Study Sponsor does not request return of the sample, it will be disposed >90 days following the study completion. Arrangements may be made for extended storage as necessary, at the Sponsor's request and expense.

XVII. Quality Assurance

- The study is conducted in accordance with Microchem Laboratory's Quality Management System and 40 CFR Part 160 and will undergo a full quality assurance review. All protocol amendments will be fully recorded and reported, as well as any deviations from the protocol.

XVIII. References

- *Annual Book of ASTM Standards*, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, Designation E1053, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- *Annual Book of ASTM Standards*, Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, Designation E1482, current edition. American Society for Testing Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.
- *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 960.09 Germicidal and Detergent Sanitizing Action of Disinfectants. Revised 2013.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing. February 2018.
- U.S. Environmental Protection Agency, Frequent Questions for the 2018 Series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. 2019.
- Guidance Document – Disinfectant Drugs. Health Canada. January 2018.
- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. January 2014.

PROTOCOL (cont.)

Virucidal Efficacy of a Test Substance for Use on Inanimate, Nonporous Surfaces

Protocol Number: P2847

Specific Testing Parameters to be completed by the Study Sponsor/Representative - all fields need to be completed before testing may commence

Test Substance Name	DoxyKlor DK5G
Test Substance Lot Numbers	06120-1/LCL, 06120-2/LCL, ~~06120-3/LCL~~ (1)
Manufacture Date(s)	6/01/2020, 6/01/2020, 6/01/2020
Expiration Date(s)	NA
Test Substance Shipment Status	☒ Use test substance already present at Microchem. ☐ Test substance will be shipped. Estimated arrival date, if known:
Test Substance Storage	☐ Room temperature (default for all packages unless otherwise advised) ☒ 2 – 8 °C ☐ Other:
Test Substance Hazards	☒ None known ☐ SDS attached ☐ Other:
Test Substance Active Ingredient	☐ Alcohol ☐ Iodophor ☐ Peracetic Acid ☐ Peroxide ☐ Phenol ☐ Quaternary Ammonia ☐ Sodium Hypochlorite ☒ Other: Chlorine dioxide
Active Ingredient Level	☒ At or below Lower Certified Limit (LCL) ☐ At or below nominal
Active Ingredient Concentration as submitted (for neutralization information only, not for chemical characterization)	0.045 % by weight
Test Substance Dilution	☒ Ready to Use (RTU) ☐ Dilution ratio: (e.g. 1 oz per gallon)
Dilution to be made	☒ N/A ☐ Dilute by adding _____ test substance to _____ diluent (please specify volumes to be used for dilution, e.g. 1 ml to 127 ml diluent) Note, an equivalent dilution may be made unless otherwise noted
Test Substance Diluent (Not applicable for RTU products)	☐ 200 ppm autoclave sterilized Tap Water (range is 180-210 ppm) ☐ 400 ppm AOAC Synthetic Hard Water (range is 360-420 ppm) ☐ 375 ppm OECD Hard Water (range is 338 – 394 ppm) ☐ Other:
Spray Instructions	☐ Not applicable, liquid product ☒ Spray carriers with 5 sprays ☐ Spray carriers for _____ seconds Distance from carrier: ☒ 4-6" ☐ 6-8" ☐ Other: _____

Page 10 of 12 Template # TFO007-1.0

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(1) Per Study Sponsor's email communication, lot number lined through was an additional sample provided. Only two need for testing. VNE 10AUG2020 & NM 10AUG2020

PROTOCOL (cont.)

Virucidal Efficacy of a Test Substance for Use on Inanimate, Nonporous Surfaces

Protocol Number: P2847

Continuation of Specific Testing Parameters to be completed by the Study Sponsor/Representative - all fields need to be completed before testing may commence

Organic Soil Load	☒ No additional organic soil load supplementation, virus will be tested as propagated (<5% organic challenge). Organic soil level will be reported ☐ 5% fetal bovine serum supplementation ☐ Other:
Contact Time(s)	10 minutes *Note: The contact time includes a range of ±5 seconds for carrier manipulation*
Exposure Temperature	☒ Room Temperature (18-25 °C) ☐ 20 ± 2 °C ☐ Other:
Number of Test and Plate Recovery Control Carriers Per Lot of Test Substance	☑ 1 ☐ 2 ☐ Other:
EPA 40 CFR Part 160.31(d) requires testing facility management to assure that the test, control, and reference substances have been appropriately tested for identity, strength, purity, stability and uniformity, as applicable.	Applicable identity, strength, purity, stability, and uniformity testing has been or will be completed prior to efficacy testing: ☒ Yes ☐ No Performed under 40 CFR Part 160 regulations? ☒ Yes ☐ No Stability testing of the formulation has been or will be completed prior to efficacy testing or concomitantly with efficacy testing: ☒ Yes ☐ No Performed under 40 CFR Part 160 regulations? ☒ Yes ☐ No If no is marked, compliance status will be noted in the GLP compliance statement in the final report.
Certificate of Analysis (CoA)	☒ CoA for each lot provided. CoA will be appended in the final report. ☐ CoA will not be provided.
Protocol Modifications	☒ Testing to be performed as outlined in the protocol. ☐ The following protocol modifications are to be performed:
Regulatory Agency(s) that report may be submitted to	☒ EPA ☐ Health Canada

Additional Instructions:

Page 11 of 12 Template # TFC007-1.0

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PROTOCOL (cont.)

XVIII. Authorized Personnel

Due to Microchem Laboratory confidentiality policy, study information will only be released to the Study Sponsor/Sponsor Representative who has signed the protocol unless otherwise noted in writing. Please list any additional personnel authorized to receive information regarding this study.

1. Peter Wood - PI Industries
2. Israel Kravzov - PI Industries
3. _____
4. _____

XIX. Protocol Approval

"I, the Study Sponsor/Sponsor Representative, have read and understand the study protocol. By signing this protocol I am certifying that the information and parameters accurately describe the test(s) to be completed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR Part 160. I have also read, understand and agree to the terms and conditions listed in the protocol."

Study Sponsor/Sponsor Representative Signature Approving Protocol

Jeremy D. Malone
Study Sponsor/Sponsor Representative Printed Name

Study Sponsor/Sponsor Representative Signature

7/09/2020
Date

jeremy@springregulatory.com
Email Address

512-922-8401
Phone

Microchem Laboratory Study Director

Victoria Zavala
Study Director Printed Name

Study Director Signature

10AUG2020
Date

CERTIFICATE OF ANALYSIS

10366 Roselle St. Suite C • San Diego, CA 92121 • 858-535-9979

Certificate of Analysis

This analysis was conducted in compliance with 40 CFR 160 as part of Expert Chemical Analysis, Inc. Study Number 6530-01.

Name:	DoxyKlor DK5G
Lot Number:	06120-1/LCL
Date of Manufacture:	06-01-20
Expiration Date:	06-01-21
Dates of Analysis:	06-01-20
By:	PI Industries, Inc.

Test	Result
Active Ingredient(s)	
Chlorine Dioxide	**0.04385 %**

Jim Polansky
Study Director
Expert Chemical Analysis, Inc.

06-12-20
Date

Moises Ramirez
Quality Assurance Manager
Expert Chemical Analysis, Inc.

06-12-20
Date

The raw data generated during analysis has been reviewed by the Quality Assurance Unit. The raw data confirm the results as listed above.

Study No. 6530-01

CERTIFICATE OF ANALYSIS (cont.)

10366 Roselle St. Suite C • San Diego, CA 92121 • 858-535-9979

Certificate of Analysis

This analysis was conducted in compliance with 40 CFR 160 as part of Expert Chemical Analysis, Inc. Study Number 6530-01.

Name:	DoxyKlor DK5G
Lot Number:	06120-2/LCL
Date of Manufacture:	06-01-20
Expiration Date:	06-01-21
Dates of Analysis:	06-01-20
By:	PI Industries, Inc.

Test	Result
Active Ingredient(s)	
Chlorine Dioxide	**0.04365 %**

Jim Polansky
Study Director
Expert Chemical Analysis, Inc.

06-12-20
Date

Moises Ramirez
Quality Assurance Manager
Expert Chemical Analysis, Inc.

06-12-20
Date

The raw data generated during analysis has been reviewed by the Quality Assurance Unit. The raw data confirm the results as listed above.

Study No. _6530-01_____