FINAL STUDY REPORT

Study Title
AOAC Germicidal Spray Products as Disinfectants Test

Product Identity
DoxyKlor DK5G
Lots: 06120-1/LCL, 06120-2/LCL, 06120-3/LCL

Test Microorganism
Salmonella enterica ATCC 10708

Data Requirements
U.S. EPA OCSPP 810.2200

Author
L. Natalia Galvan, B.S.
Study Director

Study Completion Date
16NOV2020

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

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

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

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**

Agent/Submitter: **Jeremy D. Malone**

Title: **Consultant, Spring Regulatory Sciences**

Date: **11/16/2020**

Signature:

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets U.S. Environmental Protection Agency's Good Laboratory Practice Standards and requirements for 40 CFR § 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: L. Natalia Galvan, B.S.

Title: Study Director

Signature: _____ Date: 16 NOV 2020

Study Sponsor

Company: PI Industries, Inc.

Name: **Jeremy D. Malone**

Title: **Consultant**

Signature: _____ Date: **11/16/2020**

Submitter

Company: **Spring Regulatory Sciences**

Name: **Jeremy D. Malone**

Title: **Consultant**

Signature: _____ Date: **11/16/2020**

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	02SEP2020	02SEP2020
Draft Report	10NOV2020	10NOV2020	10NOV2020
Final Report	13NOV2020	13NOV2020	13NOV2020

Signature: _____ Date: 16 NOV 2020

Name: Aaron Daniel, B.M.S.
Title: Specialist I, Quality Assurance

PERSONNEL INVOLVED IN THE STUDY

Study Director

Name: L. Natalia Galvan, B.S.
Title: Analyst I

Professional or Supervisory Personnel

Name: Hillary Johnson, M.S.
Title: Team Lead, Disinfectants and Sanitizers

Name: Hunter Cmerek, B.S.
Title: Analyst I

Name: Nathaniel Garza, B.S.
Title: Analyst I

Name: Andrea Armeriv, B.S.
Title: Associate Analyst

TABLE OF CONTENTS

FINAL STUDY REPORT....1

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS....2

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT....3

QUALITY ASSURANCE STATEMENT....4

PERSONNEL INVOLVED IN THE STUDY....5

TABLE OF CONTENTS....6

FINAL STUDY REPORT SUMMARY....7

STUDY DATES....8

TEST SUBSTANCE....8

TESTING SYNOPSIS....8

PROTOCOL CHANGES....9

TEST OBJECTIVE....9

TEST PROCEDURE....9

STUDY CONTROLS....11

SUCCESS CRITERIA....13

CALCULATIONS AND STATISTICAL ANALYSIS....14

STUDY RECORD AND TEST SUBSTANCE RETENTION....15

RESULTS....16

STUDY CONCLUSION....19

REFERENCES....20

PROTOCOL AMENDMENT....21

PROTOCOL....22

CERTIFICATE OF ANALYSIS....35

FINAL STUDY REPORT SUMMARY

Study Title: AOAC Germicidal Spray Products as Disinfectants Test

Study Identification Number: GLP2435

Test Microorganism: *Salmonella enterica* ATCC 10708

Test Substance: DoxyKlor DK5G
Lots: 06120-1/LCL, 06120-2/LCL, 06120-3/LCL

Test Substance Dilution: Ready to use liquid test substance

Organic Soil Load: No organic soil load incorporated into the test inoculum

Carrier Type: 18 mm × 36 mm glass slide

Number of Carriers Per Lot: 60

Contact Time: 10 minutes

Exposure Temperature: Ambient Temperature (22.5°C-23.2°C)
49.5-50.6% Relative Humidity

Neutralizer: Letheen Broth with 0.2% Sodium Thiosulfate (20.0 ml)

Study Results:

Test Date	Microorganism	Number Confirmed Positive Carriers/Total Number Tested			Carrier Control Avg Log_{10} CFU/Carrier
		Lot: 06120-1/LCL	Lot: 06120-2/LCL	Lot: 06120-3/LCL	
02SEP2020	*Salmonella enterica ATCC 10708*	0/60	0/60	0/60	4.32

STUDY DATES

Study Initiation Date:	17AUG2020
Experimental Start Date/Time:	02SEP2020 / 1134
Experimental Termination Date/Time:	11SEP2020 / 1142
Study Completion Date:	16NOV2020

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

Lot: 06120-3/LCL

Active Ingredients (concentration):	Chlorine Dioxide (0.04308%)
Date of Manufacture:	01JUN2020
Date Received:	20AUG2020
Expiration Date:	01JUN2021

Form: Ready to use liquid test substance

Storage Conditions: Ambient room temperature under fluorescent lighting.

Test Substance Preparation
The test substance was used as received. On the day of testing, a Sponsor provided spray bottle filled with approximately 500 ml of test substance was primed by spraying several times prior to initiation of testing.

TESTING SYNOPSIS

Upon reading results, Lot: 06120-2/LCL of DoxyKlor DK5G had one presumptive positive tube demonstrating growth by visible turbidity. Upon Gram staining, the morphology of the presumptive positive tube was Gram negative cocci, whereas the target stained as Gram negative rods. The presumptive positive tube was therefore confirmed to not be the target microorganism.

PROTOCOL CHANGES

Protocol Amendment(s)

Protocol P2880 was 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, 06120-2/LCL, and 06120-3/LCL is 01JUN2021.

All remaining testing parameters were followed as stated in the protocol.

Protocol Deviation(s)

No protocol deviations to the approved protocol were made for this study.

TEST OBJECTIVE

The purpose of this study was to document the efficacy of the test substance against the test system (microorganism) under the parameters specified in protocol P2880.

TEST PROCEDURE

Test System and Media

Test System:	*Salmonella enterica* ATCC 10708 received from the American Type Culture Collection (ATCC).
Subculture/ Neutralization Broth:	Letheen Broth with 0.2% Sodium Thiosulfate (20.0 ml)
Agar Medium:	Tryptic Soy Agar

Preparation of the Test Culture

A cryovial of frozen organism was thawed and mixed using a vortex type mixer. A 0.010 ml aliquot of the organism was inoculated into a tube containing 10.0 ml of AOAC Synthetic Broth. This culture was gently vortex mixed and incubated for 24 hours ± 2 hours at 36 °C ± 1 °C.

Three daily transfers were made by transferring 0.010 ml of the culture into 10.0 ml aliquot of sterile AOAC Synthetic Broth and incubated for 24 hours ± 2 hours at 36 °C ± 1 °C.

The test culture was initiated by transferring 0.010 ml of the daily transfer culture into four 20 × 150 mm tubes, each containing 10.0 ml sterile AOAC Synthetic Broth and incubated for 48 – 54 hours at 36 °C ± 1 °C. Exact incubation conditions present in Table 6.

The test cultures were vortex mixed for 4 seconds and allowed to dwell at room temperature for ≥ 10 minutes. The upper portion of the mixed cultures was removed, leaving behind any debris or clumps, and pooled in an appropriate vessel.

For the purposes of achieving carrier counts within the range of the study, the test culture was diluted to 1:2 using AOAC Synthetic Broth.

Supplementation of Test Culture with Organic "Soil" Load
No organic soil load was used in the study.

Preparation of Test Carriers
The glass slides were dipped in 70-95% ethyl alcohol (reagent alcohol) to remove oil and film. The carriers were thoroughly rinsed using tap water followed by two rinses in deionized water. The carriers were wiped using Kimwipes or other lint-free cloth or wipe. The carriers were visually screened for defects and only those carriers without visible defects were autoclave sterilized for use in testing. Using a biosafety cabinet, the carriers were aseptically transferred using sterile forceps into individual sterile Petri dishes matted with two sterile 9 cm round filter paper.

Carrier Inoculation with Test Culture
The sterile carriers were inoculated with 0.010 ml of test culture using a calibrated positive displacement pipette and spread to approximately 1 $inch^2$ of each test carrier without allowing the spread inoculum to touch the edges of the carrier. Each carrier was immediately covered after inoculation.

Inoculated carriers were transferred to an incubator and dried at 36.0°C at 40% relative humidity for 40 minutes until visibly dry. The inoculated carriers were used within 2 hours of drying.

Treatment of Carriers with Test Substance
Dry inoculated test carriers were horizontally oriented in the Petri dishes and sequentially treated at 20 second intervals appropriate to ensure careful and aseptic handling. Each carrier was sprayed 5 times with the test substance from a distance of 4 – 6 inches and approximately a 45° angle from the test carrier to the spray nozzle. When the first carrier was treated, a calibrated digital timer was started to measure the contact time. After treatment, carriers were covered and left to dwell undisturbed for the duration of the contact time. After the contact time (10 minutes) for each carrier had elapsed, the excess disinfectant was drained and each carrier was transferred to a 25 × 150 mm tube containing 20.0 ml of Letheen Broth with 0.2% Sodium Thiosulfate using sterile forceps. During draining the carriers did not touch the Petri dish or filter paper.
All tubes were capped and shaken after each carrier neutralization to ensure the entire carrier made contact with the subculture/neutralization broth.

The procedure was repeated until all carriers had been exposed to each test substance lot for the specified contact time and harvested into the subculture/neutralization broth.

STUDY CONTROLS

Neutralization Confirmation

Three sterile, uninoculated carriers, per lot of test substance, were treated with test substance, at 30 second intervals, identically as in the efficacy portion of the test (i.e., five sprays from a distance of 4 – 6 inches at approximately a 45° angle from carrier to spray nozzle, and a calibrated digital timer was started to measure the contact time). As the contact time elapsed (10 minutes), the carriers were sequentially transferred using sterile forceps into a tube containing 20.0 ml of subculture/neutralization broth which represented the neutralization confirmation test tubes. All tubes were capped and shaken to ensure entire carrier came in contact with neutralizer. A series of 10-fold dilutions of the test culture were performed in 9 ml of phosphate buffered dilution water (PBDW) such that a 0.100 ml volume of the dilution targeted 10-100 CFU. This inoculum was plated in duplicate to verify the number of CFU present. The neutralization confirmation test tubes were inoculated with 0.100 ml volumes of the prepared inoculum. The neutralization confirmation control was performed using multiple carrier replicates and inoculated with different dilutions of the prepared inoculum.

Enumeration of Inoculated Test Carriers

After the inoculated carriers had dried, prior to the start of the test, three carriers were randomly selected and were transferred into individual tubes containing 20.0 ml of subculture/neutralization broth. These carriers represented the carrier density at the beginning of the test. Similarly, following the conclusion of the test, three additional carriers were randomly selected and were transferred into individual tubes containing 20 ml of subculture/neutralization broth. These carriers represented the carrier density at the end of the test. The six (three pre test and three post test) carrier density subculture/neutralization broth tubes were vortex mixed for 120 seconds, as timed by a certified digital timer.
The 20.0 ml volume from the subculture/neutralization broth tubes from each set of three test tubes (pre test and post test) were pooled prior to enumeration. The pooled cultures (60.0 ml) were serially diluted in PBDW out to the 10^{-2} dilution. A 0.100 ml volume of the undiluted pool, the 10^{-1}, and the 10^{-2} dilutions were plated, in duplicate, using standard spread plating techniques, representing the 10^{-1}, 10^{-2}, and 10^{-3} dilution per ml of the carrier set. This step was performed within 2 hours of vortex mixing of the subculture/neutralization tubes.

Carrier Sterility Control

An uninoculated carrier was harvested into a 25 × 150 mm tube containing 20.0 ml of subculture/neutralization broth and incubated alongside the test.

Viability Control

An inoculated, untreated test carrier was harvested into a 25 × 150 mm tube containing 20.0 ml of subculture/neutralization broth and incubated alongside the test.

Subculture/Neutralization Sterility Control

A 25 × 150 mm tube containing 20.0 ml of subculture/neutralization broth was incubated alongside test.

Media Sterility Controls

All media sterility controls were performed on each day of testing.

A 0.1 ml aliquot of PBDW was added to sterile growth agar and incubated alongside test to confirm sterility of the serial dilution media at the time of test.

A 0.1 ml aliquot of Synthetic Broth was added to sterile growth agar and incubated alongside test to confirm sterility of culture diluent at the time of test.

A plate containing only sterile growth agar used in this study was incubated alongside test to confirm sterility of media at the time of test.

Test Microorganism Purity Control

The test culture used in this study was subcultured onto growth agar medium and incubated alongside the test to morphologically confirm the presence of target microorganism and absence of contaminant microorganism.

Incubation of Tubes and Control Plates

Test tube racks were shaken thoroughly prior to transfer to the incubator. All tubes and plates were incubated at 35.7°C for 47 hours and 7 minutes.

Confirmation of Positive Tubes Following Incubation

Tubes were assessed for the presence of growth by visual observation of turbidity of the subculture/neutralization broth. One tube for Lot: 06120-2/LCL showing visible turbidity was documented as a presumptive positive pending confirmation.
The presumptive positive tube and the viability tube were streaked onto growth agar media and incubated at 35.5-35.7°C for 19 hours and 28 minutes for confirmation of the presence of target microorganism.
Presumptive positive plates were read following incubation. Plates were stored at 4.8-5.1°C for 6 days until gram stained. A Gram stain was performed using representative colonies from the presumptive positive streak plate and the viability control. The morphology of the cells and the Gram stain results, as observed by microscopy, are reported on Table 3.

SUCCESS CRITERIA

The experimental success (controls) criteria follow:

- The test microorganism must demonstrate a concentration of at least 1.0×10^4 CFU/Carrier corresponding to a mean log density of 4.0 and not above 3.2×10^5 CFU/Carrier corresponding to a mean log density of 5.5.
- The subculture/neutralization broth sterility control tube demonstrates no growth.
- The carrier sterility control subculture/neutralization broth tube demonstrates no growth.
- The viability control subculture/neutralization broth tube demonstrates growth.
- At least one neutralization confirmation inoculum dilution demonstrates an average concentration of ≤100 CFU.
- The neutralization confirmation test subculture/neutralization broth tube corresponding to the inoculum average concentration of ≤100 CFU demonstrates growth.
- The media sterility controls demonstrate no growth.
- The test microorganism purity control plate demonstrates the presence of the target microorganism and absence of contaminant microorganisms.

The Environmental Protection Agency performance criteria for disinfection follow:

- If 1 or less non-control subculture/neutralization test tubes are confirmed positive for growth after incubation, then efficacy is demonstrated by the test substance under the conditions evaluated.
- If 2 or more non-control subculture/neutralization test tubes are confirmed positive for growth after incubation, then efficacy is not demonstrated by the test substance under the conditions evaluated.

Retesting guidance for disinfection follows:

- When a test passes and the $\log_{10}$ density of the test carriers is above 5.5, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test carriers is below 4.0, retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is below 4.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is above 5.5, retesting may be conducted.

CALCULATIONS AND STATISTICAL ANALYSIS

The following were calculations used in the study. Calculation variables were adjusted based on volumes and dilutions used.

$$\frac{(\text{Average CFU for } 10^{-x}) + (\text{Average CFU for } 10^{-y}) + (\text{Average CFU for } 10^{-z})}{10^{-x} + 10^{-y} + 10^{-z}} = \text{CFU/ml of Control Carriers}$$

where 10^{-1}, 10^{-2}, and 10^{-3} were the dilutions used

CFU/Carrier of Control Carriers = [(CFU/ml) × 20.0 ml]

$$\text{Control Carrier Mean} = \frac{(\text{Log}_{10}\text{ CFU/Carrier Pooled Pre Carriers} + \text{Log}_{10}\text{ CFU/Carrier Pooled Post Carriers})}{\text{Log Density} \qquad 2}$$

Neutralization Confirmation Inoculum = (CFU on plate 1 + CFU on plate 2) / 2

Statistical Analysis
No statistical analysis was performed.

STUDY RECORD AND TEST SUBSTANCE 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 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.

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: Carrier Enumeration Control Results

Test Microorganism	Test Date	Carrier	CFU/Carrier	Log_{10} Density	Mean Log_{10} Density
Salmonella enterica ATCC 10708	02SEP2020	Pre Treatment	1.78×10^4	4.25	4.32
		Post Treatment	2.43×10^4	4.39	

CFU = colony forming unit

Table 2: Test Results

Test Microorganism	Test Substance	Test Date	Number of Carriers Tested	Number of Test Tubes Showing Growth	Number of Test Tubes Confirmed as Test Organism
Salmonella enterica ATCC 10708	DoxyKlor DK5G Lot:06120-1/LCL	02SEP2020	60	0	0
	DoxyKlor DK5G Lot:06120-2/LCL		60	1	0*
	DoxyKlor DK5G Lot:06120-3/LCL		60	0	0

Table 3: Gram Stain Results

Test Microorganism	Test Substance	Date Performed	Colony Tested	Gram Stain	Cell Morphology	Positive Confirmed
Salmonella enterica ATCC 10708	DoxyKlor DK5G Lot: 06120-2/LCL	11SEP2020	Viability	Negative (pink)	Rods	N/A
			Positive #1		Cocci	No

*Presumptive positive confirmed to be a contaminant and not the target microorganism.

RESULTS (cont.)

Table 4: Neutralization Confirmation Results

Test Microorganism	Test Substance	Test Date	Plate Counts (CFU)	Average Inoculum Concentration (CFU)	Neutralization Test Result
Salmonella enterica ATCC 10708	DoxyKlor DK5G Lot: 06120-1/LCL	02SEP2020	12 / 13	12.5	+
			105 / 101	103	+
			>300 / >300	>300	+
	DoxyKlor DK5G Lot: 06120-2/LCL		12 / 13	12.5	+
			105 / 101	103	+
			>300 / >300	>300	+
	DoxyKlor DK5G Lot: 06120-3/LCL		12 / 13	12.5	+
			105 / 101	103	+
			>300 / >300	>300	+

Neutralization confirmation requirement met for all lots as demonstrated by positive result in at least one neutralization control tube when inoculated with an average CFU of ≤100.

Table 5: Control Results

Control Parameter	Test Date 02SEP2020
Carrier Sterility Control	No Growth Observed
Viability Control	Growth Observed
Subculture/Neutralization Sterility Control	No Growth Observed
PBDW Sterility Control	No Growth Observed
Culture Diluent Sterility Control	No Growth Observed
Growth Agar Sterility Control	No Growth Observed
Test Microorganism Purity Control	Pure- Target Microorganism circular, raised, shiny/mucoid, cream/tan colonies

RESULTS (cont.)

Table 6: Organism Propagation Conditions Test Date: 02SEP2020

Test Culture	Transfer Date and Time	Incubation Temperature Range	Culture Incubation Time
Initial thawed culture	28AUG2020 / 0942	35.7°C – 35.8°C	23 hours 52 minutes
Daily Transfer #2	29AUG2020 / 0934		23 hours 53 minutes
Daily Transfer #3	30AUG2020 / 0927		24 hours 1 minute
Final Test Culture	31AUG2020 / 0928		48 hours 14 minutes

STUDY CONCLUSION

Test substance DoxyKlor DK5G (Lots: 06120-1/LCL, 06120-2/LCL, 06120-3/LCL) was tested against *Salmonella enterica* ATCC 10708. A total of 60 contaminated carriers were exposed to each lot of the test substance for a contact time of 10 minutes at a test temperature of 22.5°C-23.2°C at 49.5-50.6% Relative Humidity and then chemically neutralized.

Following a 10 minute contact time, DoxyKlor DK5G Lots: 06120-1/LCL, disinfected 60 out of 60 carriers; Lot: 06120-2/LCL disinfected 60 out of 60 carriers against target microorganism as noted in Testing Synopsis; and Lot: 06120-3/LCL disinfected 60 out of 60 carriers.

Under the conditions of this assay, DoxyKlor DK5G Lots: 06120-1/LCL, 06120-2/LCL, 06120-3/LCL) met the requirements stated in the U.S. EPA Product Performance Test Guidelines – Disinfectants for Use on Environmental Surfaces as outlined in OCSPP 810.2200 and the success criteria detailed in the approved protocol.

The study was carried out in compliance with the approved protocol, all experimental controls met the established acceptance criteria, and there were no circumstances that may have affected the quality or the integrity of the data unless otherwise noted in the "Protocol Changes" and "Testing Synopsis" sections of this report.

REFERENCES

- "Association of Official Analytical Chemists, International." *AOAC Official Method 961.02*. Germicidal Spray Products as 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 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.
- Guidance Document – Disinfectant Drugs. Health Canada. January 2020.
- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. January 2020.
- U.S. Environmental Protection Agency, Frequent Questions for the 2018 series 810 – Product Performance Test Guidelines: Antimicrobial Efficacy Test Guidelines. 2019.

PROTOCOL AMENDMENT

MICROCHEM LABORATORY

Protocol Amendment for Protocol P2880, Study ID Number GLP2435

Protocol Amendment #1

Protocol P2880 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, 06120-2/LCL, and 06120-3/LCL is 01JUN2021.

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

Study Sponsor / Sponsor Representative (signature) — Date Signed: 10/26/2020

Jeremy D. Malone
Study Sponsor / Sponsor Representative (print)

Study Director (signature) — Date Signed: 26OCT2020

L. Natalia Galvan
Study Director (print)

PROTOCOL

MICROCHEM LABORATORY

Protocol Number: P2880 GLP Study ID: GLP2435

NCG 17AUG2020

AOAC Germicidal Spray Products as Disinfectants Test

Test Microorganism(s)
Salmonella enterica ATCC 10708

Data Requirement
U.S. EPA OCSPP 810.2200

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

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

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

Author
L. Natalia Galvan, B.S

Date
05AUG2020

PROTOCOL (cont.)

I. Introduction

This document details the materials and procedure for evaluating the efficacy of a spray disinfectant using the AOAC Germicidal Spray Products as Disinfectants Test in accordance with Good Laboratory Practice Standards (GLPS) stipulated by 40 CFR 160. This document also explains the terms and conditions of testing.

II. Purpose

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

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

The United States Environmental Protection Agency (US EPA) requires specific antimicrobial claims made for disinfectants sold in the United States to be supported by relevant test systems (microorganisms) as outlined in the United States Environmental Protection Agency 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 must 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:

- 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: 27AUG2020
Proposed Experimental Termination Date: 01SEP2020

VII. Procedure for the Identification of the 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. Following testing, the microorganism identity of positive test replicates is confirmed following the appropriate macroscopic, microscopic, and biochemical assays. All studies are assigned a unique identification number which is labeled on the test and control vessels, racks, trays, etc. Additionally, Standard Operating Procedures are also in place for the receipt, storage, and usage tracking of all test and control substances utilized in testing. These procedures are followed to identify and document the test system.

VIII. Test System (Microorganism)

Salmonella enterica ATCC 10708 received from the American Type Culture Collection (ATCC).

PROTOCOL (cont.)

IX. Procedure

Preparation of the Test Substance

- The test substance is used per Sponsor request.
- If a dilution of the test substance is requested by the Sponsor, the diluted test substance is used within three hours of preparation.
  - Unless otherwise requested by the Sponsor, if a dilution of the test substance is required, a ≥1.0 ml or ≥1.0 g of the test substance is used for preparation using volumetric glassware. For liquid products, a v/v dilution is prepared and for solids, a w/v dilution is prepared.
  - If synthetic hard water is requested as the diluent, it is prepared following Microchem Laboratory Standard Operating Procedures for the specific water type. The final hardness range is -10% to +5% of the specified hardness.
  - If tap water is requested as the diluent, the water is autoclave or filter sterilized prior to use. The water hardness is determined on the day of testing and adjusted to the hardness range if necessary. The hardness range is -10% to +5% of the specified hardness.
- If requested by the Study Sponsor, approximately 500 ml of the test substance is transferred by sterile disposable serological pipette, or other means as appropriate, into a decontaminated spray bottle.
  - Alternatively, the sponsor may provide the test substance in ready to use spray bottles, in which case, no transfer of test substance is necessary.
- Spray bottles containing the test substance are primed by spraying prior to treatment of carriers.

Preparation of Test Carriers

- Sterile 18 × 36 mm glass slides free from scratches, chips, or cracks are utilized as the test carrier.
- The carriers are cleaned by dipping in 70-95% ethyl alcohol (ethanol, reagent alcohol) or Isopropyl alcohol to remove oil and film.
- The carriers are thoroughly rinsed using tap water followed by two rinses in deionized water.
- The slides are wiped using a lint free cloth and visually screened for scratches, chips, or cracks. Defective carriers are discarded and not used for testing.
- Screened carriers are placed on a drying rack, covered in aluminum foil and autoclave sterilized on a fast/dry cycle for at least 20 minutes at approximately 121°C
- Following sterilization, the carriers may be placed into a 36±1°C incubator, oven or chamber to dry. Alternatively, the carriers may be dried at room temperature.
- Inside a biosafety cabinet, sterile carriers are aseptically transferred using sterile forceps to individual Petri dishes matted with two pieces of sterile 9cm round filter paper (Whatman No. 2, or equivalent).

Preparation of Test Culture

- A cryovial of frozen microorganism is thawed and mixed using a vortex type mixer. A 0.010 ml volume of the microorganism is inoculated into a tube containing 10 ml of sterile AOAC Synthetic Broth. The culture is gently vortex mixed and incubated for 24 hours ± 2 hours at 36°C ± 1°C.
- Subsequent daily transfers (≤5) are made by transferring 0.010 ml of the most recent daily transfer culture into 20 × 150 mm tubes containing 10 ml sterile AOAC Synthetic Broth and incubated for 24 hours ± 2 hours at 36°C ± 1°C. Only one daily transfer is required prior to initiation of the test culture.
- A test culture is initiated by transferring 0.010 ml of the most recent daily transfer culture into an appropriate number of 20 × 150 mm tubes, each containing 10 ml sterile AOAC Synthetic Broth and incubated for 48 – 54 hours at 36°C ± 1°C.
- Test cultures are vortex mixed for 3 – 4 seconds and allowed to dwell at room temperature for ≥ 10 minutes.
- The upper portion of the mixed culture(s) is removed, leaving behind any debris or clumps, and pooled in an appropriate vessel(s).

PROTOCOL (cont.)

- For the purpose of achieving carrier counts within the range of the study, dilution or concentration using centrifugation (e.g., 5000 g for 20 min) of the final test culture may be performed using the culture medium used to generate the test culture. Manipulation of the final test culture should be made prior to the addition of the organic soil load.

Supplementation of Test Culture with Organic "Soil" Load

- If requested by the Study Sponsor, an organic soil load is added to the manipulated test culture.
- The test culture is swirled gently to thoroughly mix.

Carrier Inoculation with Test Culture

- Using a calibrated positive displacement pipette, a 0.01 ml volume of test culture is spread on to approximately 1 in^2 of each test carrier without allowing the test culture to touch the edges of the carrier, and each carrier is covered immediately after inoculation.
  - Culture is periodically mixed while inoculating carriers.
  - Extra carriers should be inoculated in case any carriers are compromised during testing.
- Inoculated carriers are dried at 36°C ± 1°C in an incubator or other temperature and humidity controlled chamber for 30 – 40 minutes with only visibly dry carriers used for the test. The temperature and relative humidity conditions for carrier drying will be recorded and included in the final study report.
- Carriers are used within 2 hours of the ending dry time.

Treatment of Carriers with Test Substance

- Test exposure conditions (including temperature and relative humidity) will be recorded upon initiating and concluding the treatment of carriers.
- Dry test carriers are horizontally oriented in the Petri dish and sequentially treated at intervals appropriate to ensure careful and aseptic handling.
- Each carrier is sprayed with the test substance for the Study Sponsor specified time or with the Study Sponsor specified number of sprays/pumps at the Study Sponsor requested distance approximately 45° from the test carrier to the spray nozzle. When the first carrier is treated, a calibrated timer is started to measure the contact time. After treatment, carriers are covered and left to dwell undisturbed for the duration of the contact time.
- After the contact time for each carrier has elapsed, each carrier is transferred to a 25 × 150 mm tube containing 20 ml of the appropriate subculture/neutralization broth using sterile forceps. The excess disinfectant is drained from the carrier without touching the petri dish or filter paper.
- The tubes containing carriers are recapped and shaken to ensure the entire carrier has made contact with the subculture/neutralization broth.
- The procedure is repeated until all protocol specified efficacy carriers have been exposed to the test substance for the specified contact time and harvested into subculture/neutralization broth.
- If neutralization of the test substance is a concern, a secondary neutralization transfer from the primary neutralizer may be performed. Within 25 – 60 minutes of the initial transfer, the carriers are transferred into a second subculture tube containing a 20 ml aliquot of the subculture medium, that may contain appropriate neutralizer, using sterile forceps. Carriers are transferred in the same order as in the test, however, the transfers do not need to be timed.

PROTOCOL (cont.)

Neutralization Confirmation

- A sterile uninoculated carrier is treated per Study Sponsor instruction identically as in the test. A calibrated timer is started when the carrier is treated to measure the contact time.
- After the contact time has elapsed, the carrier is transferred to a 25 × 150 mm tube containing 20 ml of the appropriate subculture/neutralization broth using sterile forceps.
  - This tube represents the primary neutralization confirmation test tube.
  - If neutralization of the test substance is a concern and if performed in the treatment of carriers with the test substance, a secondary neutralization transfer from the primary neutralizer may be performed. Within 25 – 60 minutes of the initial transfer, the carrier is transferred into a second subculture tube containing a 20 ml aliquot of the subculture medium that may contain appropriate neutralizer using sterile forceps.
- A series of 10-fold dilutions of the test culture are performed in 9 ml of phosphate buffered dilution water (PBDW) such that a 0.1 ml volume of the prepared inoculum targets 10 – 100 CFU. This inoculum is plated in duplicate to verify the number of CFU present.
- The neutralization confirmation test tubes (primary and secondary, if applicable) are inoculated with a 0.1 ml volume of the prepared inoculum.
- The neutralization confirmation control may be performed using multiple carrier replicates and inoculated with different dilutions of the prepared inoculum.
- If more than one concentration of test substance is assayed, only the most concentrated dilution of the test substance will be evaluated in this control.
- If more than one contact time is requested, this control may be performed using the shortest requested contact time only.

Enumeration of Control Carriers

- After the inoculated carriers have dried, prior to the start of the test, three carriers are randomly selected and are transferred into individual tubes containing 20 ml of subculture/neutralization broth. These carriers represent the carrier density at the beginning of the test.
- Similarly, following the conclusion of the test, three additional carriers are randomly selected and are transferred into individual tubes containing 20 ml of subculture/neutralization broth. These carriers represent the carrier density at the end of the test.
- The six (three pre test and three post test) carrier density subculture/neutralization broth tubes are vortex mixed for 120 seconds ± 5 seconds, as measured by a calibrated timer.
- After vortex mixing, the subculture/neutralization broth tubes from each set of three test tubes (pre test and post test) are pooled prior to enumeration.
  - The total pooled volume for each set should be 60 ml.
- The pooled cultures are briefly vortexed then, enumerated by performing serial 10-fold dilutions in 9 ml PBDW and a 0.1 ml aliquot of the appropriate dilutions are plated in duplicate using standard pour and/or spread plating techniques.
  - For example, the pooled cultures are diluted out to the 10^{-2} dilution. 0.1 ml of the 10^{-0}, 10^{-1} and the 10^{-2} dilution are plated representing the 10^{-1}, 10^{-2}, and 10^{-3} dilution per ml of the carrier set.
  - This step is performed within 2 hours of vortex mixing of the pre-test and post-test subculture/neutralization tubes.

Carrier Sterility Control

- An uninoculated carrier is harvested into a 25 × 150 mm tube containing 20 ml of subculture/neutralization broth and incubated alongside the test.

Viability Control

- An inoculated, untreated test carrier is harvested into a 25 × 150 mm tube containing 20 ml of subculture/neutralization broth and incubated alongside the test.

PROTOCOL (cont.)

Subculture/Neutralization Sterility Control

- A 25 × 150 mm tube containing 20 ml of each subculture/neutralization broth utilized in testing is incubated alongside the test.

Media Sterility Controls

- A 0.1 ml volume of PBDW is added to sterile growth agar and incubated alongside the test to confirm sterility of the serial dilution media at the time of test.
- A 0.1 ml volume of culture diluent is added to sterile growth agar and incubated alongside the test to confirm sterility of culture diluent at the time of test, if applicable.
- A 0.1 ml volume of the organic soil load utilized in testing is added to sterile growth agar and incubated alongside the test to confirm soil sterility at the time of test, if applicable.
- A 0.1 ml volume of test substance diluent is added to sterile growth agar and incubated alongside the test to confirm sterility of test substance diluent at the time of test, if applicable.
- A plate containing only sterile growth agar used in this study is incubated alongside the test to confirm sterility of media at the time of test.

Test Microorganism Purity Control

- The test culture used in this study is subcultured onto growth agar medium and incubated alongside the test to morphologically confirm the presence of target microorganism and absence of contaminant microorganism.

Incubation of Tubes and Enumeration and Control Plates

- Test tube racks are shaken thoroughly prior to transfer to the incubator.
- All tubes and plates are incubated at 36°C ± 1°C for 48 hours ± 2 hours.

Confirmation of Positive Tubes Following Incubation

- Tubes are assessed for the presence of growth by visual observation of turbidity and/or a colorimetric result of the subculture/neutralization broth.
  - Test materials may be stored at 2-8°C for up to 7 days if results are not read immediately following incubation.
- The number of tubes showing visible turbidity or a colorimetric change are documented as presumptive positive pending confirmation.
  - If a colorimetric subculture/neutralization broth is used, but the test system does not produce a colorimetric change and no positive tubes are observed, ≥ 20% of the negative tubes are confirmed to be a negative result by plating on growth media.
- For 10 carrier assays or if the number of positive carriers in a 60 carrier test is less than 12, all presumptive positive tubes and the viability control are streaked onto the appropriate growth agar for confirmation of the presence of target microorganism.
  - If the number of tubes demonstrating growth is is greater than 12 in a 60 carrier test, ≥20% of presumptive positive tubes and the viability control are streaked onto the appropriate growth agar for confirmation of the presence of target microorganism.
- All confirmatory plates are incubated for 18 – 24 hours at 36°C ± 1°C.
  - Confirmatory plates may be stored at 2-8°C for up to 7 days if results are not read immediately following incubation.
- The colony morphology of the viability control and the presumptive positives is noted in the raw data, if applicable.
  - If any additional morphology is observed, they are noted in the raw data.

PROTOCOL (cont.)

- A Gram stain is performed using representative colonies from the presumptive positive streak plate(s) and viability control, if applicable.
  - If multiple colony morphologies are observed, Gram stain analysis is performed on each unique colony morphology.
  - The morphology of the cells and the Gram stain results, as observed by microscopy, are recorded in the raw data for each colony tested.
- Other appropriate biochemical analysis may be performed for confirmation of the presence of the test microorganism.
- The number of confirmed positive tubes is documented in the raw data and reported along with any confirmed contaminants.

X. Calculations

The following are calculations to be used in the study. Calculation variables may be adjusted based on volumes and dilutions used.

$$\frac{\text{(Average CFU for } 10^{-x}) + \text{(Average CFU for } 10^{-y}) + \text{(Average CFU for } 10^{-z})}{10^{-x} + 10^{-y} + 10^{-z}} = \text{CFU/ml of Control Carriers}$$

where 10^{-x}, 10^{-y}, and 10^{-z} are examples of dilutions that may be used. For the calculation above, dilutions yielding counts up to 300 are used and dilutions resulting in plate counts of 0 are included in the calculation. Counts greater than 300 may be documented as >300 or as TNTC and are not included in calculations.

[(CFU/ml of Control Carriers) × 20 ml] = CFU/Carrier of Control Carriers

$$\text{Control Carrier Mean} = \frac{\text{(Log}_{10}\text{ CFU/Carrier Pooled Pre Carriers + Log}_{10}\text{ CFU/Carrier Pooled Post Carriers)}}{2}$$

Log Density

Neutralization Confirmation Inoculum =(CFU on Plate 1 + CFU on plate 2) / 2

XI. Proposed Statistical Analysis

Not applicable.

XII. Methods for the Control of Bias

Not applicable.

PROTOCOL (cont.)

XIII. Success Criteria

The experimental success (controls) criteria follow:

- The test microorganism must demonstrate a concentration of at least 1.0×10^4 CFU/Carrier corresponding to a mean log density of 4.0 and not above 3.2×10^5 CFU/Carrier corresponding to a mean log density of 5.5.
- The subculture/neutralization broth sterility control tube demonstrates no growth.
- The carrier sterility control subculture/neutralization broth tube demonstrates no growth.
- The viability control subculture/neutralization broth tube demonstrates growth.
- At least one neutralization confirmation inoculum dilution demonstrates an average concentration of ≤100 CFU.
- The neutralization confirmation test subculture/neutralization broth tube corresponding to the inoculum average concentration of ≤100 CFU demonstrates growth.
- The media sterility controls demonstrate no growth.
- The test microorganism purity control plate demonstrates the presence of the target microorganism and absence of contaminant microorganisms.

If any controls do not meet the specified experimental success criteria, testing may be repeated at the discretion of the Study Director under the same study protocol.

XIV. Product Performance Criteria

The Environmental Protection Agency performance criteria for disinfection follow:

- If 1 or less non-control subculture/neutralization test tubes are confirmed positive for growth after incubation, then efficacy is demonstrated by the test substance under the conditions evaluated.
- If 2 or more non-control subculture/neutralization test tubes are confirmed positive for growth after incubation, then efficacy is not demonstrated by the test substance under the conditions evaluated.

Retesting guidance for disinfection follows:

- When a test passes and the $\log_{10}$ density of the test carriers is above 5.5, no retesting is necessary.
- When a test passes and the $\log_{10}$ density of the test carriers is below 4.0, retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is below 4.0, no retesting is necessary.
- When a test fails and the $\log_{10}$ density of the test carriers is above 5.5, retesting may be conducted.

XV. Reporting

Results are reported accurately and fully, in accordance with Environmental Protection Agency 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 EPA 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

- "Association of Official Analytical Chemists, International." *AOAC Official Method 961.02.* Germicidal Spray Products as 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 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.
- Guidance Document – Disinfectant Drugs. Health Canada. January 2020.
- Guidance Document – Safety and Efficacy Requirement for Hard Surface Disinfectant Drugs. Health Canada. January 2020.
- U.S. Environmental Protection Agency, Frequent Questions for the 2018 series 810 – Product Performance Test Guidelines: Antimicrobial Efficiency Test Guidelines. 2019.

PROTOCOL (cont.)

AOAC Germicidal Spray Products as Disinfectants

Protocol Number: P2880

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 Batch Numbers	06120-1/LCL, 06120-2/LCL, 06120-3/LCL
Manufacture Date(s)	6/1/2020, 6/01/2020, 6/01/2020
Expiration Date(s)	NA
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 =_____ total parts (please specify volumes to be used for dilution, eg. 1 ml to 127 ml diluent to equal 128 parts or 1 ml to 128 ml diluent to equal 128 parts) Note, an equivalent dilution may be made unless otherwise noted
Test Substance Diluent (Not applicable for RTU products)	☐ 200 ppm autoclave sterilized Tap Water (hardness range is 180-210 ppm) ☐ 400 ppm AOAC Synthetic Hard Water (hardness range is 360-420 ppm) ☐ 375 ppm OECD Hard Water (hardness range is 338 – 394 ppm) ☐ Other:
Test Substance Spray Bottles (Not applicable if test substance supplied in spray bottle for testing)	☐ Test substance supplied in bulk, transfer to Sponsor supplied spray bottle and use Sponsor supplied spray nozzle ☒ Test substance supplied in bulk, transfer to laboratory supplied spray bottle and use laboratory spray nozzle

PROTOCOL (cont.)

AOAC Germicidal Spray Products as Disinfectants

Protocol Number: P2880

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	☒ None ☐ 5% fetal bovine serum ☐ Other:
Contact Time(s)	10 minutes *Note: Contact times of ≤ 1 minute include a ± 3 seconds. Contact times of > 1 minute include a ± 5 seconds.*
Exposure Temperature	☒ Room Temperature ☐ Other:
Number of Test Carriers Per Batch	☐ 10 ☒ 60 ☐ Other:
Spray Instructions	☒ Spray carriers with 5 sprays or until thoroughly wet ☐ Spray carriers for _____ seconds Spray setting: ☒ Spray ☐ Jet/Stream ☐ Mist Distance from carrier: ☒ 4-6" ☐ 6-8" ☐ Other: _____
Neutralization/Subculture Broth	☒ Microchem to determine. Sponsor authorizes pre-test neutralization confirmation assay to be conducted to determine appropriate neutralizer, if needed. Additional fees may apply per price quotation. ☐ Use:
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 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 for either question, compliance status will be noted in the GLP compliance statement in the final report.
Certificate of Analysis (CoA)	☒ CoA for each batch provided. CoA will be appended in the final report. ☐ CoA will not be provided.
Test Substance Shipment Status	☒ Use test substance already present at Microchem. ☐ Test substance will be shipped. Estimated arrival date, if known:
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

PROTOCOL (cont.)

XIX. 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. _____

XX. Protocol Approval

"I, the Study Sponsor, 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 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 — 8/07/2020 Date

Jeremy@SpringRegulatory.com
Email address

512-922-8401
Phone

Microchem Laboratory Study Director

L. Natalia Galvan
Study Director Printed Name

Study Director Signature — 17AUG2020 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_____

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:	06120-3/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	**0.04308 %**

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_____