

## FINAL STUDY REPORT

## STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Newcastle disease virus

## PRODUCT IDENTITY

1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975)

> TEST GUIDELINE OCSPP 810.2200

PROTOCOL NUMBER

CHE003060816.NEW

## <u>AUTHOR</u>

Mary J. Miller, M.T. Study Director

## STUDY COMPLETION DATE

August 29, 2016

### PERFORMING LABORATORY

Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121

**SPONSOR** 

Chemours Company FC, LLC Experimental Station 402/5232B 200 Powder Mill Road P.O. Box 8352 Wilmington, DE 19803

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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 sec. 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:	Chemours Company FC, LLC	
Company Agent:		
	Title	
		Date:
	Signature	

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## GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) regulations set forth in 40 CFR Part 160.

Submitter:

Date:

Sponsor:\_\_\_\_\_

Date:\_\_\_\_\_

Study Director: Mary D. Miller Date: 8-29-16 Mary J. Miller, M.T.

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## QUALITY ASSURANCE UNIT SUMMARY

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

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. This study has been performed in accordance to standard operating procedures and the study protocol. In accordance with Good Laboratory Practice regulation 40 CFR Part 160, the Quality Assurance Unit maintains a copy of the study protocol and standard operating procedures and has inspected this study on the date(s) listed below. Studies are inspected at time intervals to assure the integrity of the study. The findings of these inspections have been reported to Management and the Study Director.

Phase Inspected Date of Phase Inspection		Date Reported to Study Director	Date Reported to Management	
Critical Phase Audit: Preparation of Test Substance	udit: ēst July 28, 2016 July 28, 2016		July 28, 2016	
Draft Report	August 16, 2016	August 16, 2016	August 29, 2016	
Final Report	August 25, 2016	August 25, 2016	August 29, 2010	

Quality Assurance Specialist;	F	Date: 8-29-16
	1	



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## STUDY PERSONNEL

STUDY DIRECTOR:

Mary J. Miller, M.T.

Professional Personnel Involved:

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Shanen Conway, B.S. Katherine A. Paulson, M.L.T. Matthew Cantin, B.S. Erica Flinn, B.A. Miranda Peskar, B.S. Kyle Kuras, B.S.

- Manager, Virology Laboratory Operations
- Lead Virologist
- Virologist
- Virologist
- Associate Virologist
- Microbiologist

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## STUDY REPORT

## **GENERAL STUDY INFORMATION**

Protocol Number: CHE003060816.NEW

- Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces
- Project Number: A21347
- Protocol Number: CHE003060816.NEW

Sponsor: Chemours Company FC, LLC Experimental Station 402/5232B 200 Powder Mill Road P.O. Box 8352 Wilmington, DE 19803

Testing Facility:Accuratus Lab Services1285 Corporate Center Drive, Suite 110Eagan, MN 55121

## TEST SUBSTANCE IDENTITY

Test Substance Name: Virkon™ S

Lot/Batch(s): Lot # 1502260974 and Lot # 1502260975

## **Test Substance Characterization**

Test substance characterization as to identity, strength, purity, solubility and composition, as applicable, according to 40 CFR, Part 160, Subpart F [160.105], was documented prior to its use in the study. The Test Substance Certificate of Analysis Reports may be found in Attachments I-II.

## STUDY DATES

Date Sample Received:June 21, 2016Study Initiation Date:June 29, 2016Experimental Start Date:July 28, 2016 (Start time: 11:17 a.m.)Experimental End Date:August 4, 2016 (End time: 9:35 a.m.)Study Completion Date:August 29, 2016



## OBJECTIVE

The objective of this study was to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure was to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA).

## SUMMARY OF RESULTS

Test Substance:	1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975)
Dilution:	1:200 defined as 4.8 g ± 0.03 g of test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water
Virus:	Newcastle disease virus, ATCC VR-108 Strain B1, Hitchner or Blacksburg
Exposure Time:	1 minute
Exposure Temperature:	5°C (5.0°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975) met the performance requirements specified in the study protocol. The results indicate <b>complete inactivation</b> of Newcastle disease virus under these test conditions as required by the U.S. EPA.

## TEST SYSTEM

1. <u>Virus</u>

The B1, Hitchner or Blacksburg strain of Newcastle disease virus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-108). The stock virus was prepared by collecting the allantoic fluid from inoculated ten day old fertilized, embryonated chicken eggs. The allantoic fluid was clarified by centrifugation at approximately 1500 RPM for 10 minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at  $\leq$ -70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot NDV-44) was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain a 5% fetal bovine serum organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Newcastle disease virus on chicken embryo fibroblast cells.

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## 2. Indicator Cell Cultures

Cultures of chicken embryo fibroblast (CEF) cells were originally obtained from Charles River. The cells were propagated by Accuratus Lab Services personnel. The cells were seeded into multiwell cell culture plates and maintained at  $36-38^{\circ}$ C in a humidified atmosphere of 5-7% CO<sub>2</sub>. On the day of testing, the cells were observed as having proper cell integrity and confluency, and therefore, were acceptable for use in this study.

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

3. Test Medium

The test medium used in this study was Minimum Essential Medium (MEM) supplemented with 2% (v/v) heat-inactivated fetal bovine serum (FBS), 10  $\mu$ g/mL gentamicin, 100 units/mL penicillin, 2.5  $\mu$ g/mL amphotericin B, 5% (v/v) tryptose phosphate broth and 2.0 mM L-glutamine.

## TEST METHOD

1. Preparation of Test Substance

1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975) were tested at a 1:200 dilution defined as 4.8 g  $\pm$  0.03 g of test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water as requested by the Sponsor. Lot # 1502260974 was prepared using 4.80 g test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water and Lot # 1502260975 was prepared using 4.81 g test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water and Lot # 1502260975 was prepared using 4.81 g test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water. Each lot of test substance was in solution as determined by visual observation and used on the day of preparation. The prepared test substance was equilibrated to the exposure temperature prior to use.

The 400 ppm AOAC Synthetic Hard Water was prepared using 12.6 mL of Solution I and 12.0 mL of Solution II. The total volume of hard water was brought to approximately 3 liters using sterile deionized water. The 400 ppm hard water was prepared, titrated (at 400 ppm) and used on the day of testing.

2. Preparation of Virus Films

Films of virus were prepared by spreading 200  $\mu$ L of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C in a relative humidity of 50% until visibly dry (20 minutes).

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## 3. Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus was separated from the test substance by filtration through Sephadex LH-20 gel. On the day of testing, Sephadex columns were prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns were then ready to be used in the assay.

- 4. <u>Input Virus Control</u> (TABLE 1) On the day of testing, the stock virus utilized in the assay was titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.
- 5. Treatment of Virus Films with the Test Substance (TABLE 1)

For each lot of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 1 minute at  $5^{\circ}$ C ( $5.0^{\circ}$ C). The virus films were completely covered with the test substance. Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates ( $10^{-1}$  dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

6. <u>Treatment of Dried Virus Control Film</u> (TABLE 1)

One virus film was prepared as previously described (paragraph 2). The virus control film was exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 1 minute at 5°C ( $5.0^{\circ}$ C). Just prior to the end of the exposure time, the virus control was scraped with a cell scraper and at the end of the exposure time the virus mixture was immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrate ( $10^{-1}$  dilution) was then titered by 10-fold serial dilution and assayed for infectivity.

7. Cytotoxicity Controls (TABLE 2)

A 2.00 mL aliquot of the use dilution of each lot of the test substance was filtered through a Sephadex column and the filtrate was diluted serially in medium and inoculated into chicken embryo fibroblast cell cultures. Cytotoxicity of the chicken embryo fibroblast cell cultures was scored at the same time as the virus-test substance and virus control cultures.

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## Assay of Non-Virucidal Level of Test Substance (Neutralization Control) (TABLE 3)

Each dilution of the neutralized test substance (cytotoxicity control dilutions) was challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, was retained. Dilutions that showed virucidal activity were not considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures was inoculated with a 100  $\mu$ L aliquot of each dilution in quadruplicate. A 100  $\mu$ L aliquot of low titer stock virus (approximately 32 infectious units) was inoculated into each cell culture well and the indicator cell cultures were incubated along with the test and virus control plates.

## 9. Infectivity Assays

The chicken embryo fibroblast cell line, which exhibits cytopathic effect (CPE) in the presence of Newcastle disease virus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100  $\mu$ L of the dilutions prepared from test and control groups. The input virus control was inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell culture labware. The cultures were scored periodically for seven days for the absence or presence of CPE, cytotoxicity, and for viability.

10. Statistical Methods: Not applicable

## PROTOCOL CHANGES

### **Protocol Amendments:**

No protocol amendments were required for this study.

## **Protocol Deviations:**

No protocol deviations occurred during this study.

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## DATA ANALYSIS

## **Calculation of Titers**

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

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

## **Calculation of Log Reduction**

Dried Virus Control Log<sub>10</sub> TCID<sub>50</sub> – Test Substance Log<sub>10</sub> TCID<sub>50</sub> = Log Reduction

## STUDY ACCEPTANCE CRITERIA

## U.S. EPA Submission

A valid test requires 1) that at least 4  $\log_{10}$  of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

## RECORD RETENTION

## **Study Specific Documents**

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121 for a minimum of five years following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. The original data includes, but is not limited to, the following:

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

## Test Substance Retention

The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test substance.



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## REFERENCES

- 1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1053-11.
- 2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1482-12.
- 3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
- 4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces - Efficacy Data Recommendations, September 4, 2012.
- 5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
- 6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.

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## STUDY RESULTS

Results of tests with 1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975), diluted 1:200 defined as  $4.8 \text{ g} \pm 0.03 \text{ g}$  of test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water, exposed to Newcastle disease virus in the presence of a 5% fetal bovine serum organic soil load at 5°C (5.0°C) for 1 minute are shown in Tables 1-3. All cell controls were negative for test virus infectivity.

The titer of the input virus control was 6.00 log<sub>10</sub>. The titer of the dried virus control was 5.00 log<sub>10</sub>. Following exposure, test virus infectivity was not detected in the virus-test substance mixture for either lot at any dilution tested ( $\leq 0.50 \log_{10}$ ). Test substance cytotoxicity was not observed in either lot at any dilution tested ( $\leq 0.50 \log_{10}$ ). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at  $\leq 0.50 \log_{10}$  for both lots. Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer was  $\geq 4.50 \log_{10}$  for both lots.

## STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, 1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975), diluted 1:200 defined as 4.8 g  $\pm$  0.03 g of test substance + 1000 mL of 400 ppm AOAC Synthetic Hard Water, demonstrated complete inactivation of Newcastle disease virus following a 1 minute exposure time at 5°C (5.0°C) as required by the U.S. EPA.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.

The use of the Accuratus Lab Services name, logo or any other representation of Accuratus Lab Services without the written approval of Accuratus Lab Services is prohibited. In addition, Accuratus Lab Services may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the expressed written permission of Accuratus Lab Services.



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## **TABLE 1: Virus Controls and Test Results**

## Effects of 1502260974SN (Lot # 1502260974) and 1502260975SN (Lot # 1502260975) Following a 1 Minute Exposure to Newcastle Disease Virus Dried on an Inanimate Surface

Dilution	Input Virus Control	Dried Virus Control	Newcastle disease virus + Lot # 1502260974	Newcastle disease virus + Lot # 1502260975
Cell Control	0 0	0000	0000	0000
10 <sup>-1</sup>	+ +	++++	0000	0000
10 <sup>-2</sup>	+ +	++++	0000	0000
10 <sup>-3</sup>	+ +	++++	0000	0000
10-⁴	++	++++	0000	0000
10 <sup>-5</sup>	+ +	0 + + 0	0000	0000
10 <sup>-6</sup>	0 +	0000	0000	0000
10 <sup>-7</sup>	0 0	NT	NT	NT
TCID <sub>50</sub> /100 μL	10 <sup>6.00</sup>	10 <sup>5.00</sup>	≤10 <sup>0.50</sup>	≤10 <sup>0.50</sup>

(+) = Positive for the presence of test virus

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

(NT) = Not tested

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## TABLE 2: Cytotoxicity Control Results

## Cytotoxicity of 1502260974SN and 1502260975SN on Chicken Embryo Fibroblast Cell Cultures

Dilution	Cytotoxicity Control Lot # 1502260974	Cytotoxicity Control Lot # 1502260975
Cell Control	0000	0000
10 <sup>-1</sup>	0000	0000
10 <sup>-2</sup>	0000	0000
10 <sup>-3</sup>	0000	0000
10⁴	0000	0000
10 <sup>-5</sup>	0000	0000
10 <sup>-6</sup>	0000	0000
TCD₅₀/100 µL	≤10 <sup>0.50</sup>	≤10 <sup>0.50</sup>

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

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## TABLE 3: Neutralization Control Results

## Non-Virucidal Level of the Test Substance (Neutralization Control)

Dilution	Test Virus + Cytotoxicity Control Lot # 1502260974	Test Virus + Cytotoxicity Control Lot # 1502260975
Cell Control	0000	0000
10 <sup>-1</sup>	++++	++++
10 <sup>-2</sup>	+ + + +	++++
10 <sup>-3</sup>	+ + + +	++++
10⁴	++++	++++
10 <sup>-5</sup>	++++	++++
10 <sup>-6</sup>	++++	++++

(+) = Positive for the presence of test virus after low titer stock virus added (neutralization control)

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

Results of the non-virucidal level control indicate that the test substance was neutralized at a TCID<sub>50</sub>/100  $\mu$ L of ≤0.50 log<sub>10</sub> for both lots.

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## \_\_\_\_\_

ATTACHMENT I: Test Substance Certificate of Analysis – Lot # 1502260974

## Haviland Products Company

# Certificate of Analysis

Item: VIRKON S Lot #: 1502260974 Date: March 13, 2015

## Analysis:

<u>Test:</u>	Specification:	R	<u>Result:</u>
Appearance	Pale Yellow/OrangeFree Flowing Powder Free of Foreign Particles	i.	Passes
pH at 1%	2.20 - 2.65		2.44
Available Oxyg	gen % 9.75 – 10.25		9.82

The above is a true copy of the analysis of this lot.

ky Maton

Dwight Meston

EXACT COPY INITIALS DATE 8-29-16

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## ATTACHMENT II: Test Substance Certificate of Analysis – Lot # 1502260975

## Haviland Products Company

# Certificate of Analysis

Item: VIRKON S

Lot #: 1502260975

**Date:** March 13, 2015

## Analysis:

Test:	Specification:	Result:
Appearance	Pale Yellow/OrangeFree Flowing Powder Free of Foreign Particles	Passes
pH at 1%	2.20 - 2.65	2.31
Available Oxy	gen % 9.75 – 10.25	9.76

The above is a true copy of the analysis of this lot.

· Kup Watan

Dwight Meston

EXACT COPY INITIALS M DATE 8-29-16

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(For Laboratory Use Only) Accuratus Lab Services Project # Test Substance Tracking # TSQ 03 140 6-21-16 ICRA



PROTOCOL

## Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

Virus: Newcastle disease virus

#### PROTOCOL NUMBER

CHE003060816.NEW

#### PREPARED FOR/SPONSOR

Chemours Company FC, LLC Experimental Station 402/5232B 200 Powder Mill Road P.O. Box 8352 Wilmington, DE 19803

#### PREPARED BY/TESTING FACILITY

Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121

DATE

June 8, 2016

EXACT COPY INITIALS CONDATE 8-29-10

PROPRIETARY INFORMATION

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#### Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

#### PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA), Health Canada and Australian Therapeutic Goods Administration (TGA).

#### TEST SUBSTANCE CHARACTERIZATION

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identify, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

### SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the <u>proposed</u> experimental start date is June 28, 2016. Verbal results may be given upon completion of the study with a written report to follow on the <u>proposed</u> completion date of July 26, 2016. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, because of failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services.

The Sponsor is responsible for any rejection of the final report by the regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

#### JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. Each agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The chicken embryo fibroblast cell line, which supports the growth of the Newcastle disease virus, will be used in this study. The experimental design in this protocol meets these requirements.

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- Proprietary Information -

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#### TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. At the end of the exposure time, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

#### STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

The inoculated carriers are exposed to the test substance for the Sponsor specified exposure time. At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

#### VIRUS

The B1, Hitchner or Blacksburg strain of Newcastle disease virus to be used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-108). Stock virus is prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at  $\leq$  -70°C until the day of use. Alternate methods of viral propagation may be utilized based on the growth requirements of the virus. The propagation method will be specified in the raw data and in the report. On the day of use the appropriate number of aliquots are removed, thawed, combined (if applicable) and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine will be adjusted to yield the percent organic soil load requested.

#### INDICATOR CELL CULTURES

Cultures of chicken embryo fibroblast (CEF) cells were originally obtained from Charles River. The cells are propagated by Accuratus Lab Services personnel. The cells are seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub>. The confluency of the cells will be appropriate for the test virus. CEF cells obtained from an alternate, reputable source may be used. The source of the cells will be specified in the final report.

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

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#### TEST MEDIUM

The test medium used for this assay is Minimum Essential Medium (MEM) supplemented with 0-10% (v/v) heat inactivated fetal bovine serum. The medium may also be supplemented with one or more of the following: 10  $\mu$ g/mL gentamicin, 100 units/mL penicillin, 2.5  $\mu$ g/mL amphotericin B, 1.0-2.0 mM L-glutamine, and 0.5 – 5  $\mu$ g/mL trypsin. The composition of the test medium may be altered based on the virus and/or cells. The composition of the medium will be specified in the raw data and in the report.

#### PREPARATION OF TEST SUBSTANCE

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be preequilibrated to the desired test temperature if applicable.

#### PREPARATION OF VIRUS FILMS

Films of virus will be prepared by spreading 200 µL of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

One dried virus film per batch of test substance will be assayed unless otherwise requested.

#### TEST METHOD

#### Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

#### Input Virus Control

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

#### Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10<sup>-1</sup> dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

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#### **Treatment of Dried Virus Control Film**

The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate (10<sup>-1</sup> dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

#### **Cytotoxicity Control**

A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

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

Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 100  $\mu$ L aliquot of each dilution in quadruplicate. A 100  $\mu$ L aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates.

#### Infectivity Assays

The CEF cell line, which exhibits cytopathic effect (CPE) in the presence of Newcastle disease virus, will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with 100  $\mu$ L of the dilutions prepared from test and control groups. The input virus control will be inoculated in duplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO<sub>2</sub> in sterile disposable cell cultures will be iniccubation time the cultures will be recorded on the raw data worksheets; only the results from the final observations will be reported.

#### DATA ANALYSIS

#### **Calculation of Titers**

Viral and cytotoxicity titers will be expressed as  $-\log_{10}$  of the 50 percent titration endpoint for infectivity (TCID<sub>50</sub>) or cytotoxicity (TCD<sub>50</sub>), respectively, as calculated by the method of Spearman Karber.

I	(	Sum of % mortality at each dilution	-05	x (logarithm of dilution)	
- Log of 1st difution modulated -	U	100	) = 0.5		

#### **Calculation of Log Reduction**

Dried Virus Control Log<sub>10</sub> TCID<sub>50</sub> – Test Substance Log<sub>10</sub> TCID<sub>50</sub> = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

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#### PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

#### STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

#### U.S. EPA, Health Canada, and Australian TGA Submission

A valid test requires 1) that at least 4  $\log_{10}$  of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

If the test substance fails to meet the test acceptance criteria and the dried virus control fails to meet the control acceptance criteria, the study is considered valid and no repeat testing is necessary, unless requested by the Sponsor.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number.

#### FINAL REPORT

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, and a conclusion as it relates to the purpose of the test. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

#### PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

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#### TEST SUBSTANCE RETENTION

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be <u>discarded</u> following study completion unless otherwise requested.

#### RECORD RETENTION

#### Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

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

#### **Facility Specific Documents**

The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

- 1. SOPs which pertain to the study conducted.
- 2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
- 3. Methods which were used or referenced in the study conducted.
- 4. QA reports for each QA inspection with comments.
- 5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
- 6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

PROPOSED STATISTICAL METHODS: N/A

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#### REFERENCES

- Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1053-11.
- Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides, Antimicrobials, and Alternative Control Agents; Environmental Assessment; Hazardous Substances and Oil Spill Response, E1482-12.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations, September 4, 2012.
- 5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E.H., Lennette, D.A. and Lennette, E.T. editors. Seventh edition, 1995.
- Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
- 7. Health Canada, January, 2014. Guidance Document Disinfectant Drugs.
- 8. Health Canada, January, 2014. Guidance Document Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
- 9. Australian Therapeutic Goods Administration (TGA), February 1998. Guidelines for the Evaluation of Sterilants and Disinfectants.
- 10. Australian Therapeutic Goods Administration (TGA), February 1998. Therapeutic Goods Order No. 54: Standard for Disinfectants and Sterilants.
- 11. Australian Therapeutic Goods Administration (TGA), March 1997. Therapeutic Goods Order No. 54A: Amendment to Standard for Disinfectants and Sterilants (TGO 54).
- 12. Australian Therapeutic Goods Administration (TGA), July 2005. Draft Guidelines for the Evaluation of Household/Commercial and Hospital Grade Disinfectants.

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	(All block anotions are completed by the Spansor		to their signature u	nlose otherwise not	lod )
Lo	Test Substance (Name and Batch Number 15 \$\mathcal{P}_{226}\$\mathcal{P}_{74}\$\mathcal{S}_{20}\$\mathcal{A}_{20}\$\mathcal{P}_{226}\$\mathcal	exactly as it should appear on $\frac{99755N}{2000} \frac{P_{232}}{P_{232}} + \frac{7}{125} \frac{1}{5}$ $\frac{1000}{100} \frac{9975}{100}$ the hardest to-kill virus on you	final report): bstance Tden r label is required	ty State: Vic	kon The S
	Product Description Quaternary ammonia I lodophor	eracetic acid	odium hypochlorit	æ	
	Approximate Test Substance Active Concer 214/28 Patassium Manpressulfate; 1.5% 5 (This value is used for neutralization planning only	ntration (upon submission to A <u>ปี แค ปาใงการ์ การให้ oth</u> . This value is not intended to repre	ccuratus Lab Se	rvices): on values.)	
2	Storage Conditions	C Other			
	Hazards None known: Use Standard Precaution Material Safety Data Sheet, Attached As Follows:	ns for each product —			
	Product Preparation No dilution required, Use as received ( ✓ "Dilution(s) to be tested: <u>1:200</u> defined as (example: 1 oz/gallon) Deionized Water (Filter or Autoclave Tap Water used will be determined AOAC Synthetic Hard Water: <u>1/2</u> Other *Note: An equivalent dilution may be mage	RTU) $4.89m_3\pm0.037+100$ (amount of test substance) (a e Sterilized) rilized) - All tap water is softened and reported. PPM (3= A d ade unless otherwise requested	フロルス mount of diluent) d; the water hardn d ed per しー。 d by the Sponsor		n of -\ . -{6
	Test Virus: <u>Newcastle disease virus</u>	_			
	Exposure Time: <u>1 minute</u>	100 a a			
	Exposure Temperature: C Room temperature:	re (to be based on regulatory age °C (please specify range)	ency of submissior	1)	
	Directions for application of aerosol/spray p	products:			
	Trigger spray application: Spray carriers using 3 sprays, or until the Spray carriers using spra Aerosol spray application:	broughly wet, at a distance of 6 to ys at a distance of to	8 inches. inches/cm. (c	ircle one)	
	□ Spray carriers for seconds, or to Organic Soil Load □ 1% fetal bovine serum (minimum level th ☑ 5% fetal bovine serum □ Other	untin thoroughly wet, at a distance nat can be tested)	or to	Inches/cm.	
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#### Number of Carriers to be Tested

☑ One (typical for U.S. EPA submission)

Five (required for broad-spectrum virucidal claims for Health Canada submission)

SPRAY BOTTLES USED IN TESTING (section only applicable for spray products) To ensure expected levels of product are delivered, it is recommended that the Sponsor provide the spray bottles used in testing. Please indicate the desired source of the sprayer bottles used in testing:

Sprayer(s) and bottle(s) are provided by the Sponsor

General purpose spray bottle(s) are to be provided by Accuratus Lab Services

The spray nozzle(s) are provided by the Sponsor and general purpose bottle(s) will be provided by Accuratus Lab Services

#### REGULATORY AGENCY(S) THAT MAY REVIEW DATA

- $\square$ U.S. EPA
- ۵ Health Canada
- Therapeutic Goods Administration (Australian TGA)
- Not applicable - For internal/other use only (Efficacy result will be based on U.S. EPA requirements)

#### COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures. ⊠ Yes

No (Non-GLP or Development Study)

#### PROTOCOL MODIFICATIONS

- Approved without modification
- Approved with modification

#### PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - D Yes D No

### TEST SUBSTANCE SHIPMENT STATUS

- (This section is for informational purposes only.)
- Test Substance is already present at Accuratus Lab Services.
- Test Substance has been or will be shipped to Accuratus Lab Services. Date of expected receipt at Accuratus Lab Services: June 2016
- C Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director).

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## TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING

[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

Characterization/Stability testing is not required (For Non-GLP or Development testing only)

ÖR

Physical and Chemical Characterization (Identity, purity, strength, solubility, as applicable) of the test lots 🕱 Physical & Chemical Characterization has been or will be completed prior to efficacy testing.

- GL'P compliance status of physical & chemical characterization testing:
- Testing was or will be performed following 40 CFR Part 160 GLP regulations
   Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:

- 2 A Certificate of Analysis (C of A) may be provided for each lot of test substance. If provided, the C of A will be appended to the report.
- Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:

Test has been or will be conducted by another facility under protocol or study #:

Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.

Stability Testing of the formulation

Ì۵ Stability testing has been or will be completed prior to or concurrent with efficacy testing.

GLP compliance status of stability testing:

(GLP compliance is required by 40 CFR Part 160)

Testing was or will be performed following 40 CFR Part 160 GLP regulations Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:

Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:

Test has been or will be conducted by another facility under protocol or study #:

Stability testing was not or will not be performed prior to or concurrent with efficacy testing.

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.

()= Added per 6-23-16 email. mm 6-24-16

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APPROVAL SIGNATURES	×	
NAME:Ms. Sherrill Numberg	TITLE:Microbiology Ass	ociate Investigator
SIGNATURE: Spinill Mundring	DATE: Juss 14, 201	lo
PHONE: <u>(302) <del>695 - 8497</del></u> FAX: <u>(</u> 353 - 5つイン	302) 695 - 8680 EMAIL: <u>Sherrill.Number</u> 353・5つぞく	g@chemours.com
For confidentiality purposes, study information protocol (above) unless other individuals are	on will be released only to the sponsor/representati a specifically authorized in writing to receive study ir	ve signing the formation.
Other individuals authorized to receive in	nformation regarding this study: 🛛 🗘 S	See Attached

Accuratus Lab Services:

Carl W. Erkenbrecher, Jr.

Mary NAME: Study Director -Mary J-M. Ole Study Director SIGNATURE:

Carl. W Erkenbrecher - JRO. chemours. com

DATE: 6-29-16

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