ACCURATUS
—LAB SERVICES—
THE ANTIMICROBIAL AUTHORITY

FINAL STUDY REPORT

STUDY TITLE

Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus

PRODUCT IDENTITY

Atmosphere
Lot TC0501183 and Lot TC0501184

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

ATM002032218.FCAL

AUTHOR

Matt Cantin, B.S.
Study Director

STUDY COMPLETION DATE

June 26, 2018

PERFORMING LABORATORY

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

SPONSOR

Atmosphere Global LLC
55 West Goethe Unit 1241
Chicago, IL 60610

PROJECT NUMBER

A25603

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: Atmosphere Global LLC

Company Agent: Mel Jones

Manager of Domestic and International Operations
Title

Signature

Date: July 30, 2018

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: July 30, 2018
Mel Jones

Sponsor: _____ Date: July 30, 2018
Mel Jones

Study Director: _____ Date: 6/26/18
Matt Cantin, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus

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	June 7, 2018	June 7, 2018	June 12, 2018
Final Report	June 26, 2018	June 26, 2018	June 26, 2018

Quality Assurance Specialist: Cody Sam Date: 6/26/18

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STUDY PERSONNEL

STUDY DIRECTOR: Matt Cantin, B.S.

Professional Personnel Involved:

Shanen Conway, B.S.	- Manager, Study Director Operations
Erica Flinn, B.A.	- Manager, Virology Laboratory Operations
Mary J. Miller, M.T.	- Senior Virologist
Katherine A. Paulson, M.L.T.	- Lead Virologist
Kasey Thompson, B.S.	- Associate Virologist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus

Project Number: A25603

Protocol Number: ATM002032218.FCAL

Sponsor: Atmosphere Global LLC
55 West Goethe Unit 1241
Chicago, IL 60610

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

TEST SUBSTANCE IDENTITY

Test Substance Name: Atmosphere

Lot/Batch(s): Lot TC0501183 and Lot TC0501184

Manufacture Dates: Lot TC0501183 – May 1, 2018
Lot TC0501184 – May 1, 2018

Test Substance Characterization
Test substance characterization as to identity, strength, purity, stability and uniformity, 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: May 3, 2018
Study Initiation Date: June 5, 2018
Experimental Start Date: June 7, 2018 (Start time: 12:53 p.m.)
Experimental End Date: June 14, 2018 (End time: 5:59 a.m.)
Study Completion Date: June 26, 2018

OBJECTIVE

The purpose of this study was to evaluate the virucidal efficacy of a test substance against Feline Calicivirus, to be used as a surrogate virus for human Norovirus, 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 the U.S. Environmental Protection Agency (EPA), Health Canada and Australian Therapeutic Goods Administration (TGA).

Norovirus, a member of the *Caliciviridae* family, is a non-enveloped RNA-containing virus and is an important cause of gastroenteritis in humans. Little is known about disinfectant efficacy against this virus due to the inability to propagate the virus in-vitro. Feline Calicivirus, also a member of the *Caliciviridae* family, serves as a valuable model virus for efficacy testing of Norovirus, since these viruses share many similar characteristics and Feline Calicivirus can be propagated in cell cultures.

SUMMARY OF RESULTS

Test Substance:	Atmosphere, Lot TC0501183 and Lot TC0501184
Dilution:	1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water
Virus:	Feline Calicivirus as a surrogate virus for Norovirus
Exposure Time:	10 minutes
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	Two lots of Atmosphere (Lot TC0501183 and Lot TC0501184) met the EPA test criteria specified in the study protocol. Under these test conditions, both replicates of both lots **demonstrated a ≥3 $\log_{10}$ reduction** in titer of Feline Calicivirus.

TEST SYSTEM

1. Virus

The F-9 strain of Feline Calicivirus used for this study was obtained from the American Type Culture Collection, Manassas, VA (ATCC VR-782). The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, two aliquots of stock virus (Accuratus Lab Services lot FC2-31) were removed, thawed, combined and refrigerated until used in the assay. The stock virus culture contained 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Feline Calicivirus on Crandel Reese feline kidney cells. The cytopathic effect observed was small, rounding of the cells, with a slight granular look.

2. Indicator Cell Cultures

Cultures of Crandel Reese feline kidney (CRFK) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-94). The cells were propagated by Accuratus Lab Services personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. The confluency of the cells was appropriate for the test virus. This cell line has historically been used as the cell line for propagation and detection of Feline Calicivirus. The cultures were commercially available, were serially propagated, and were capable of showing cytopathic effect in the presence of the virus.

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.

3. Test Medium

The test medium used for this assay was Minimum Essential Medium (MEM) supplemented with 5% (v/v) heat inactivated fetal bovine serum. The medium was also supplemented with 10 μg/mL gentamicin, 100 units/mL penicillin and 2.5 μg/mL amphotericin B.

The following table lists the test and control groups, the dilutions assayed per carrier, and the number of culture wells per dilution. See text for a more detailed explanation.

SAMPLES TESTED FOR THE PRESENCE OF VIRUS		
Test or Control Group	**Dilutions Assayed Per Carrier (log_{10})**	**Culture Wells per Dilution**
Negative Controls	N/A	2-4
Input Virus Control	-4,-5,-6,-7,-8	4
Dried Virus Control (performed in duplicate)	-4,-5,-6,-7,-8	4
Test Substance – Lot #1 (performed in duplicate)	-1,-2,-3,-4	4
Test Substance – Lot #2 (performed in duplicate)	-1,-2,-3,-4	4
Cytotoxicity Control – Test Substance Lot #1	-1,-2,-3	2
Cytotoxicity Control – Test Substance Lot #2	-1,-2,-3	2
Neutralization Control – Test Substance Lot #1	-1,-2,-3	2
Neutralization Control – Test Substance Lot #2	-1,-2,-3	2

TEST METHOD

1. Preparation of the Test Substance

   Two lots of Atmosphere (Lot TC0501183 and Lot TC0501184) were tested at a 1:60 dilution defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water (1.00 mL product + 59.0 mL water) as requested by the Sponsor. The 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 ≥200 ppm unsoftened tap water was titrated (at 200 ppm) and used on the day of testing.

2. Preparation of the Virus Films

   Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottoms of six 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).

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. Input Virus Control

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

For each lot of test substance, two dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for 10 minutes at room temperature (20.0°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. Treatment of Dried Virus Control Films

Two virus films were prepared as previously described (paragraph 2). The virus control films were exposed to 2.00 mL of test medium in lieu of the test substance and held covered for 10 minutes at room temperature (20.0°C). Just prior to the end of the exposure time, the virus control films were scraped with a cell scraper and at the end of the exposure time the virus mixtures were immediately passed through a Sephadex column in the same manner as the test virus (paragraph 5). The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity. The purpose of this control is to determine the titer of the dried virus that was exposed to the test system. The average titer of the dried virus control replicates was used to calculate the log reduction in viral titer of the individual test replicates.

7. Cytotoxicity Assay

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 CRFK cell cultures. Cytotoxicity of the CRFK cell cultures was scored at the same time as the virus-test substance and virus control cultures.

8. Assay of Non-Virucidal Level of Test Substance (Neutralization Control)
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 µL aliquot of each dilution in duplicate. A 100 µL aliquot of low titer stock virus (approximately 100 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 CRFK cell line, which exhibits cytopathic effect (CPE) in the presence of Feline Calicivirus, was used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from test and virus control groups. The cytotoxicity and neutralization control dilutions were inoculated in duplicate. Uninfected indicator cell cultures (cell controls) were inoculated with test medium alone. Cultures were incubated at 31-35°C (33.1°C) in a humidified atmosphere of 5-7% CO_2 (6.2-6.4% CO_2) in sterile disposable cell culture labware. The cultures were microscopically scored periodically for seven days for the absence or presence of CPE, cytotoxicity and for viability.

10. Statistical Methods: Not applicable

PROTOCOL CHANGES

Protocol Amendment:
Per Sponsor request, this protocol is amended to include the following modifications, which will align this protocol with the February 2018 version of the 810.2000 and 810.2200 Product Performance Test Guidelines:

a. The study acceptance criteria section is updated to reflect that, for EPA submission, a valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

b. The Product Performance Test Guidelines in the references section, OCSPP 810.2000 and 810.2200, will be updated to reflect the February 2018 version of the guidelines accordingly:

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

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

c. Calculations
For Calculation of Titers section of the protocol, the calculation listed is to determine the $TCID_{50}$/ volume inoculated.

To calculate $TCID_{50}$/carrier:
(Antilog of dried virus control $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 Log_{10})

*This is the $TCID_{50}$ value calculated based on the volume inoculated per well as described in the Calculation of Titers section of the protocol.

d. The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier, in place of the most probable number (MPN) statistical method:

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

The average titer of the dried virus control replicates will be calculated and used to calculate the log reduction in viral titer of the individual test replicates.

d. The manufacture date of each product lot will be included in the report. Per the test substance labels, the manufacture date for both lots is 5/1/18.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculation of Titers

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

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

Calculation of Titer per Carrier ($TCID_{50}$/carrier)

(Antilog of dried virus control $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log of Y = the $TCID_{50}$/carrier

* = The $TCID_{50}$ value calculated based on the volume inoculated per well.

Calculation of Log Reduction

The following calculation was used to calculate the log reduction per volume inoculated per well and the log reduction per carrier:

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

The average titer of the dried virus control replicates was calculated and used to calculate the log reduction in viral titer of the individual test replicates.

STUDY ACCEPTANCE CRITERIA

U.S. EPA Submission

A valid test requires 1) that at least 4.8 log_{10} of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 log_{10} reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 log_{10} reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

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. **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.

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. American Society of Testing and Materials (ASTM). Standard Practice for Use of Gel Filtration Columns for Cytotoxicity Reduction and Neutralization, E1482-12 (Reapproved 2017).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing, February 2018.
4. 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.
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.
7. Inactivation of feline Calicivirus, a Norwalk virus surrogate, Journal of Hospital Infection (1999) 41: 51-57.
8. Virucidal Efficacy of Four New Disinfectants, Journal of the American Animal Hospital Association, Vol. 38 No. 3, May/June 2002, Pages 231-234.
9. Efficacy of Commonly Used Disinfectants for the Inactivation of Calicivirus on Strawberry, Lettuce, and Food-Contact Surface, Journal of Food Protection, Vol. 64, No. 9, 2001, Pages 1430-1434.
10. Concentration and Detection of Caliciviruses from Food Contact Surfaces, Journal of Food Protection, June 2002; 65 (6).
11. Health Canada January 2014. Guidance Document – Disinfectant Drugs.
12. Health Canada January 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
13. Australian Therapeutic Goods Administration (TGA), February 1998. Guidelines for the Evaluation of Sterilants and Disinfectants.
14. Australian Therapeutic Goods Administration (TGA), February 1998. Therapeutic Goods Order No. 54: Standard for Disinfectants and Sterilants.
15. Australian Therapeutic Goods Administration (TGA), March 1997. Therapeutic Goods Order No. 54A: Amendment to Standard for Disinfectants and Sterilants (TGO 54).
16. Australian Therapeutic Goods Administration (TGA), July 2005. Draft Guidelines for the Evaluation of Household/Commercial and Hospital Grade Disinfectants.
17. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.

STUDY RESULTS

Results of tests with two lots of Atmosphere (Lot TC0501183 and Lot TC0501184), diluted 1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water, exposed to Feline Calicivirus in the presence of a 5% fetal bovine serum organic soil load at room temperature (20.0°C) for 10 minutes are shown in Tables 1-3. All cell controls were negative for test virus infectivity.

The input titer (not dried) of the virus was 7.50 $\log_{10}$/100 µL. The titer of the dried virus control was 7.25 $\log_{10}$/100 µL (7.55 $\log_{10}$/carrier) for Replicate #1 and 6.50 $\log_{10}$/100 µL (6.80 $\log_{10}$/carrier) for Replicate #2. The average titer of the two dried virus control replicates was 7.02 $\log_{10}$/100 µL (7.32 $\log_{10}$/carrier). The average titer was used to calculate the log reduction in viral titer of each of the test replicates.

Following exposure, test virus infectivity was detected in the virus-test substance mixture for Lot TC0501183 at 3.50 $\log_{10}$/100 µL (3.80 $\log_{10}$/carrier) for Replicate #1 and 3.50 $\log_{10}$/100 µL (3.80 $\log_{10}$/carrier) for Replicate #2.

Following exposure, test virus infectivity was detected in the virus-test substance mixture for Lot TC0501184 at 3.75 $\log_{10}$/100 µL (4.05 $\log_{10}$/carrier) for Replicate #1 and 2.75 $\log_{10}$/100 µL (3.05 $\log_{10}$/carrier) for Replicate #2.

Test substance cytotoxicity was not observed in either lot at any dilution tested (≤0.50 $\log_{10}$/100 µL). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at ≤0.50 $\log_{10}$ for both lots.

Taking the cytotoxicity and neutralization control results into consideration, the reduction in viral titer, per volume inoculated per well and per carrier, for was 3.52 $\log_{10}$ for both Replicate #1 and #2 of Lot TC0501183, 3.27 $\log_{10}$ for Replicate # 1 of Lot TC0501184 and 4.27 $\log_{10}$ for Replicate # 2 of Lot TC0501184.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, Atmosphere, diluted 1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water, demonstrated a ≥3 $\log_{10}$ reduction in titer of Feline Calicivirus following a 10 minute exposure time at room temperature (20.0°C).

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

TABLE 1: Virus Control Results

Input Virus Control Results and Results of Feline Calicivirus Dried on an Inanimate Surface Following a 10 minute Exposure Time

Dilution	Input Virus Control	Dried Virus Control	
		Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0
10^{-4}	+ + + +	+ + + +	+ + + +
10^{-5}	+ + + +	+ + + +	+ + + +
10^{-6}	+ + + +	+ + + +	+ + + +
10^{-7}	+ + + +	+ + 0 +	0 0 0 0
10^{-8}	0 0 0 0	0 0 0 0	0 0 0 0
$TCID_{50}$/100 µL	$10^{7.50}$	$10^{7.25}$	$10^{6.50}$
$TCID_{50}$/carrier	NA	$10^{7.55}$	$10^{6.80}$
Average $TCID_{50}$/100 µL	NA	$10^{7.02}$	
Average $TCID_{50}$/carrier	NA	$10^{7.32}$	

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(NA) = Not applicable

TABLE 2: Test Substance Assay Results

Effects of Atmosphere (Lot TC0501183 and Lot TC0501184) Following a 10 minute Exposure to Feline Calicivirus Dried on an Inanimate Surface

Dilution	Feline Calicivirus + Lot TC0501183		Feline Calicivirus + Lot TC0501184	
	Replicate #1	Replicate #2	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
10^{-1}	+ + + +	+ + + +	+ + + +	+ + + +
10^{-2}	+ + + +	+ + + +	+ + + +	+ + + +
10^{-3}	+ + + 0	+ 0 + +	+ + + +	0 0 + 0
10^{-4}	0 0 + 0	+ 0 0 0	0 0 0 +	0 0 0 0
$TCID_{50}$/100 µL	$10^{3.50}$	$10^{3.50}$	$10^{3.75}$	$10^{2.75}$
$TCID_{50}$/carrier	$10^{3.80}$	$10^{3.80}$	$10^{4.05}$	$10^{3.05}$
Log Reduction*	3.52 $\log_{10}$	3.52 $\log_{10}$	3.27 $\log_{10}$	4.27 $\log_{10}$

(+) = Positive for the presence of test virus
(0) = No test virus recovered and/or no cytotoxicity present
(*) = This is the log reduction per volume inoculated per well and per carrier.

TABLE 3: Test Substance Cytotoxicity and Neutralization Control Results

Dilution	Cytotoxicity Control Lot TC0501183	Cytotoxicity Control Lot TC0501184	Neutralization Control Lot TC0501183	Neutralization Control Lot TC0501184
Cell Control	0 0	0 0	0 0	0 0
10^{-1}	0 0	0 0	+ +	+ +
10^{-2}	0 0	0 0	+ +	+ +
10^{-3}	0 0	0 0	+ +	+ +
TCD_{50}/100 µL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	See below	See below

(+) = 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 $TCID_{50}$/100 µL of ≤0.50 $\log_{10}$ for both lots of test substance.

ATTACHMENT I: Certificate of Analysis – Lot TC0501183

Certificate of Analysis

The analysis of this test substance was conducted in compliance with Good Laboratory Practice Standards as published in 40 CFR Part 160 as part of Accuratus Lab Services' Project Number: A25430.

Sponsor:	Atmosphere Global LLC 55 West Goethe Unit 1241 Chicago, IL 60610
Test Facility:	Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Test Substance Name:	Atmosphere
Lot/Batch:	TC0501183
Expiration Date:	September 1, 2020
Protocol Number:	ATM002030818.CHR
Date of Analysis:	May 7, 2018

EXACT COPY
INITIALS ll DATE 6-26-18

Active Ingredient (Test)	Purity Result
Quaternary Ammonia	6.88%

Study Director Emily Breen Date: 05/23/18
Emily Breen, B.A.

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

Quality Assurance Specialist: Cody Barry Date: 5/23/18

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ATTACHMENT II: Certificate of Analysis – Lot TC0501184

ACCURATUS
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Certificate of Analysis

The analysis of this test substance was conducted in compliance with Good Laboratory Practice Standards as published in 40 CFR Part 160 as part of Accuratus Lab Services' Project Number: A25430.

EXACT COPY
INITIALS ll DATE 6/26/18

Sponsor:	Atmosphere Global LLC 55 West Goethe Unit 1241 Chicago, IL 60610
Test Facility:	Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Test Substance Name:	Atmosphere
Lot/Batch:	TC0501184
Expiration Date:	September 1, 2020
Protocol Number:	ATM002030818.CHR
Date of Analysis:	May 7, 2018

Active Ingredient (Test)	Purity Result
Quaternary Ammonia	6.96%

Study Director Emily Breen Date: 05/23/18
Emily Breen, B.A.

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

Quality Assurance Specialist: Cody Dam Date: 5/23/18

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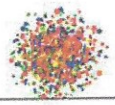

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AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	June 14, 2018
Sponsor:	Atmosphere Global LLC 55 West Goethe Unit 1241 Chicago, IL 60610
Test Facility:	Accuratus Lab Services 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus
Protocol Number:	ATM002032218.FCAL
Project Number:	A25603

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Modifications to Protocol:

Per Sponsor request, this protocol is amended to include the following modifications, which will align this protocol with the February 2018 version of the 810.2000 and 810.2200 Product Performance Test Guidelines:

a. The study acceptance criteria section is updated to reflect that, for EPA submission, a valid test requires 1) that at least 4.8 $\log_{10}$ of infectivity per carrier be recovered from the dried virus control film; 2) that a ≥3 $\log_{10}$ reduction in titer must be demonstrated; 3) if cytotoxicity is evident, at least a 3 $\log_{10}$ reduction in titer must be demonstrated beyond the cytotoxic level. Similarly, the log reduction will also take into consideration the level of neutralization; 4) that the cell controls be negative for infectivity. An efficacious product does not need to demonstrate complete inactivation at all dilutions.

b. The Product Performance Test Guidelines in the references section, OCSPP 810.2000 and 810.2200, will be updated to reflect the February 2018 version of the guidelines accordingly:

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AMENDMENT TO GLP TEST PROTOCOL

- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides – Guidance for Efficacy Testing, February 2018.
- U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces – Guidance for Efficacy Testing, February 2018.

c. Calculations
For Calculation of Titers section of the protocol, the calculation listed is to determine the $TCID_{50}$/ volume inoculated.

To calculate $TCID_{50}$/carrier:
(Antilog of dried virus control $TCID_{50}$*) x (volume inoculated per carrier/ volume inoculated per well) = Y

Log of Y = the $TCID_{50}$/carrier (Example: $10^{5.80}$ or 5.80 Log_{10})

*This is the $TCID_{50}$ value calculated based on the volume inoculated per well as described in the Calculation of Titers section of the protocol.

d. The following calculation will be used to calculate the log reduction per volume inoculated per well and the log reduction per carrier, in place of the most probable number (MPN) statistical method:

Dried Virus Control Log_{10} $TCID_{50}$ – Test Substance Log_{10} $TCID_{50}$ = Log Reduction

The average titer of the dried virus control replicates will be calculated and used to calculate the log reduction in viral titer of the individual test replicates.

d. The manufacture date of each product lot will be included in the report. Per the test substance labels, the manufacture date for both lots is 5/1/18.

Changes to the protocol are acceptable as noted.

Study Director

6/8/18
Date