ACCURATUS
—LAB SERVICES—
THE ANTIMICROBIAL AUTHORITY

FINAL STUDY REPORT

STUDY TITLE

AOAC Use-Dilution Method

Test Organism(s):

Listeria monocytogenes (ATCC 19117)

PRODUCT IDENTITY

Atmosphere
Lot TC0501183 and Lot TC0501184

TEST GUIDELINE

OCSPP 810.2200

PROTOCOL NUMBER

ATM002032218.UD.4

AUTHOR

Andrea Epperly, B.S.
Study Director

STUDY COMPLETION DATE

July 2, 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

A25619

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: Andrea Epperly Date: 7/2/18
Andrea Epperly, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: AOAC Use-Dilution Method

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: Carrier Population Control	June 14, 2018	June 14, 2018	June 14, 2018
Final Report	June 23, 2018	June 25, 2018	July 2, 2018

Quality Assurance Specialist: Jackie Kyrianki Date: 7/2/18

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STUDY PERSONNEL

STUDY DIRECTOR:	Andrea Epperly, B.S.
Professional personnel involved:	
Shanen Conway, B.S.	- Manager, Study Director Operations
Amy Backler, M.S.	- Manager, Microbiology Laboratory Operations
John Egan, B.S.	- Microbiology Laboratory Supervisor
Matthew Kranz, B.S.	- Microbiologist
Kyle Kuras, B.S.	- Microbiologist
Alicia Doberstein, B.S.	- Associate Microbiologist
Melanie Gorham, A.A.S.	- Associate Microbiologist

STUDY REPORT

GENERAL STUDY INFORMATION

Study Title: AOAC Use-Dilution Method

Project Number: A25619

Protocol Number: ATM002032218.UD.4

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

Lots: Lot TC0501183 and Lot TC0501184

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.

STUDY DATES

Date Sample Received: May 3, 2018
Study Initiation Date: June 5, 2018
Experimental Start Date: June 14, 2018 (Start time: 10:36 am)
Experimental End Date: June 18, 2018 (End time: 2:45 pm)
Study Completion Date: July 2, 2018

OBJECTIVE

The objective of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA), Health Canada, and the Australian Therapeutic Goods Administration (TGA).

SUMMARY OF RESULTS

Test Substances:	Atmosphere (Lot TC0501183, and Lot TC0501184)
Dilution:	1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water
Test Organisms:	*Listeria monocytogenes* (ATCC 19117)
Exposure Time:	10 minutes
Exposure Temperature:	20±1°C (20.0°C)
Organic Soil Load:	5% Fetal Bovine Serum
Number of Carriers:	10 per batch
Efficacy Result:	Atmosphere demonstrated efficacy of two out of two batches against *Listeria monocytogenes*, and therefore, meets the performance requirements set forth by the U.S. EPA, Health Canada and Australian Therapeutic Goods Administration following a 10 minute exposure time at 20±1°C (20.0°C) in the presence of a 5% Fetal Bovine Serum organic soil load.

STUDY MATERIALS

Test System/Growth Media

Test Organism	Designation #	Growth Medium	Incubation Parameters
Listeria monocytogenes	19117	Brain Heart Infusion Broth	35-37°C, aerobic

The test organism used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Recovery Media

Neutralizing Subculture Medium: Brain Heart Infusion Broth + 0.07% Lecithin & 0.5% Tween 80

Agar Plate Medium: Tryptic Soy Agar with 5% Sheep's Blood (BAP)

Reagents

Organic Soil Load Description: 5% Fetal Bovine Serum (FBS)

Un-softened Tap Water:
The Sponsor specified ≥200 ppm sterile un-softened tap water was titrated on the day of use for water hardness. The tap water used in testing was determined to have a 202 ppm hardness.

Carriers
Carriers were screened according to the AOAC Official Method of Analysis and all carriers positive for growth were discarded. Only penicylinders which demonstrated no growth during screening were used in this test. Stainless steel penicylinders were pre-soaked overnight in 1N NaOH, washed in water until neutral and autoclaved in deionized water. Carriers were used within three months of sterilization.

TEST METHOD

Preparation of Test Substance
An equivalent dilution of 1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water was prepared using 8.0 mL of the test substance and 472.0 mL of ≥200 ppm unsoftened tap water. Volumetric glassware was used. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 100 mm tubes, placed in a 20±1°C (20.0°C) water bath and allowed to equilibrate for ≥10 minutes prior to testing.

Preparation of Test Organism
A loopful of stock slant culture was transferred to an initial 10 mL tube of growth medium.

The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C (36.0°C). Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Three additional daily transfers were prepared inoculating a sufficient number of tubes for the final test culture. The final test culture was incubated for 48-54 hours (49 hours) at 35-37°C (36.0°C).

The test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel and mixed.

The culture was adjusted using sterile growth medium to target a spectrophotometer absorbance reading between 0.240 and 0.260 (0.252) at 620 nm. The culture was then diluted using sterile growth medium by combining 0.50 mL of test organism suspension with 49.5 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use.

Addition of Organic Soil Load
A 1.50 mL aliquot of FBS was added to 28.5 mL of prepared culture to yield a 5% fetal bovine serum organic soil load.

Contamination of Carriers
The culture was transferred to the penicylinders (after siphoning off the water) and the carriers were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were completely covered by the culture. The inoculated carriers were transferred to sterile Petri dishes matted with filter paper after tapping the carrier against the side of the container to remove excess inoculum. No more than twelve carriers were placed in each Petri dish. The carriers were dried for 38 minutes at 35-37°C (35.9-36.0°C) and at 49.4-51.2% relative humidity. Carriers were used in the test procedure within 2 hours of drying.

Exposure Conditions
Each contaminated and dried carrier was placed into a separate tube containing 10.0 mL of the test substance at its use-dilution. Immediately after placing each test carrier in the test tube, the tube was swirled using approximately 2–3 gentle rotations to release any air bubbles trapped in or on the carrier. The carriers were exposed for 10 minutes at 20.0°C. Care was taken to avoid touching the sides of the tubes. The carrier was placed into the test substance within ±5 seconds of the exposure time following a calibrated timer.

Test System Recovery
Following the Sponsor specified exposure time, each medicated carrier was transferred by wire hook at staggered intervals to 10 mL of neutralizing subculture medium and each tube was shaken thoroughly. To accomplish this, the carrier was removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant and transferred into the subculture tube. Tapping the carrier against the upper third of the tube was avoided. Care was taken to avoid excessive contact with the interior sides of the subculture tubes during transfer.

Incubation and Observation
All subculture vessels and control plates were incubated for 48±2 hours (47.5 hours) at 35-37°C (36.0°C). Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were visually examined for the presence or absence of growth.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" was performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
Concurrent with testing, the serum used for the organic soil load was cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
Concurrent with testing, a representative uninoculated carrier was added to the neutralizing subculture medium. The subculture medium containing the carrier was incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
Concurrent with testing, a representative sample of uninoculated neutralizing subculture medium was incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
One representative inoculated carrier was added to a vessel containing subculture medium. The vessel containing the carrier was incubated and visually examined for growth. The acceptance criterion for this study control is growth in the subculture medium.

Neutralization Confirmation Control
The neutralization of the test substance was confirmed concurrent with testing by exposing at least one sterile carrier to the test substance and transferring the carrier to subcultures containing 10 mL of neutralizing subculture medium as in the test. The subcultures were inoculated with a target of 10-100 colony forming units (CFU) of the test organism, incubated under test conditions and visually examined for the presence of growth. This control was performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure was run concurrently in order to enumerate the number of CFU per tube actually added.

The acceptance criterion for this study control is growth in the subculture broth following inoculation with ≤100 CFU per tube.

Carrier Population Control
Two sets of three inoculated carriers (one set prior to testing and one set following treatment) were assayed. Each inoculated carrier was individually subcultured into a tube containing 10 mL of neutralizing subculture medium and sonicated for 1 minute ±5 seconds. Tubes were contained in a beaker with water suspended in the ultrasonic cleaner such that all fluids were level. Following sonication, the contents of the three subcultured carriers were pooled (30 mL) and briefly vortex mixed. Appropriate serial ten-fold dilutions were prepared and the duplicate aliquots spread plated on agar plate medium and incubated. Following incubation, the resulting colonies were enumerated. The individual CFU per carrier set results were calculated and the Log_{10} value of each carrier set was determined. The average Log_{10} value was calculated. The acceptance criterion for this study control is a minimum average Log_{10} value of 4.0.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for label claims state that the test substance must kill the microorganism on 10 out of the 10 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations

The CFU/Carrier set in the Carrier Population Control was determined using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{[(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})] \times (\text{Volume of neutralizer})}{[10^{-x} + 10^{-y} + 10^{-z}] \times (\text{Volume plated}) \times (\#\text{ of carriers per set})}$$

Where 10^{-x}, 10^{-y}, and 10^{-z} are example dilutions that may be used

$$\text{Average } \text{Log}_{10} \text{ Carrier Population Control} = \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \ldots \text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier set
N equals number of control carrier sets

Statistical Analysis
None used.

STUDY RETENTION

Record Retention

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 final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention

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

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 964.02, Testing Disinfectants against *Pseudomonas aeruginosa* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. Association of Official Analytical Chemists (AOAC) Official Method 955.15, Testing Disinfectants against *Staphylococcus aureus* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
3. Association of Official Analytical Chemists (AOAC) Official Method 955.14, Testing Disinfectants against *Salmonella enterica*- Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
4. 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.
5. 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.
6. 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.
7. Health Canada, January, 2014. Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs.
8. Health Canada, January, 2014. Guidance Document - 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 the Standard for Disinfectants and Sterilants (TGO 54).

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including the culture purity, viability, organic soil sterility, neutralizing subculture medium sterility, carrier sterility, carrier population, and neutralization confirmation were within acceptance criteria.

For Test Results, see Table 4.

ANALYSIS

Atmosphere (Lot TC0501183) diluted 1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water, demonstrated no growth of *Listeria monocytogenes* (ATCC 19117) in any of the 10 subculture tubes following a 10 minute exposure time at 20±1°C (20.0°C) in the presence of a 5% Fetal Bovine Serum organic soil load.

Atmosphere (Lot TC0501184) diluted 1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water, demonstrated no growth of *Listeria monocytogenes* (ATCC 19117) in any of the 10 subculture tubes following a 10 minute exposure time at 20±1°C (20.0°C) in the presence of a 5% Fetal Bovine Serum organic soil load.

STUDY CONCLUSION

Under the conditions of this investigation, 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 efficacy against *Listeria monocytogenes*, as required by the U.S. EPA, Health Canada and the Australian Therapeutic Goods Administration following a 10 minute exposure time at 20±1°C (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: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results
	Listeria monocytogenes **(ATCC 19117)**
Purity Control	Pure
Viability Control	Growth
Organic Soil Sterility Control	No Growth
Neutralizing Subculture Medium Sterility Control	No Growth
Carrier Sterility Control	No Growth

TABLE 2: CARRIER POPULATION CONTROL RESULTS

Test Organism: *Listeria monocytogenes* (ATCC 19117)							
Volume Plated: 0.100 mL							
Carrier set	**Dilution Factor**				**CFU/ carrier**	**Log_{10}**	**Average Log_{10}**
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**			
Pre-testing	41, 41	8, 8	1, 1	0, 1	4.6×10^4	4.66	4.61
Post-testing	31, 36	3, 7	2, 0	0, 0	3.6×10^4	4.56	

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONFIRMATION CONTROL RESULTS

Test Substance	Test Organism	Dilution	CFU Added	Average CFU	Number of Subcultures	
					Tested	Positive
Atmosphere Lot TC0501183	*Listeria monocytogenes* (ATCC 19117)	10^{-5}	T, T	>300	1	1
		10^{-6}	60, 64	62	1	1
		10^{-7}	15, 14	15	1	1
Atmosphere Lot TC0501184	*Listeria monocytogenes* (ATCC 19117)	10^{-5}	T, T	>300	1	1
		10^{-6}	60, 64	62	1	1
		10^{-7}	15, 14	15	1	1

CFU = Colony Forming Unit
T = Too Numerous To Count (>300 colonies)

TABLE 4: TEST RESULTS

Test Substance	Test Organism	Sample Dilution	Number of Carriers		
			Exposed	Showing Growth	Confirmed As Test Organism
Atmosphere Lot TC0501183	*Listeria monocytogenes* (ATCC 19117)	1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water	10	0	0
Atmosphere Lot TC0501184	*Listeria monocytogenes* (ATCC 19117)	1:60 defined as 1 part test substance + 59 parts ≥200 ppm unsoftened tap water	10	0	0