GIBRALTAR LABORATORIES, INC.
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Study Title
EPA Non-food Contact Sanitizer Test on Peraspray

Product Identity
"Peraspray"

Data Requirement
DIS/TSS-10 Efficacy Data Requirements Supplemental Efficacy Sanitizer Test (for inanimate, non-food contact surfaces)

Author
Jozef Mastej
Microbiology Manager

Study Completion Date
02/03/2010

Testing Facility
Gibraltar Laboratories, Inc.
16 Montesano Road
Fairfield, NJ 07004

Laboratory Project Number (Study File)
GBL Study # GR2635

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B) or (C).

Company ENVIRO TECH CHEMICAL SERVICES Inc

Company Agent N/A Date Feb 10, 2010

President Michael Harvey

Title Signature

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR Part 160 with the exception that the test agent stability information, synthesis, and purity analysis, composition and other characteristics of the test product remain with the sponsor.

SUBMITTER: Tina Rodrigues — 2/11/10
Date

Tina Rodrigues
Study Submitter Name

GLP Coordinator
Study Submitter Title

SPONSOR: Enviro Tech Chemical Services, Inc.

Mike Harvey — 2-10-2010
Date

Mike Harvey
Study Sponsor Name

STUDY DIRECTOR: Jozef Mastej — 2/3/2010
Date

Jozef Mastej
Microbiology Manager

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QUALITY ASSURANCE STATEMENT

Study Title: EPA Non-food Contact Sanitizer Test on Peraspray

Study Number: GR2635

In accordance with the Good Laboratory Practice Standards (EPA 40 CFR Part 160), quality assurance audits of this study were conducted and reported to management and the study director as listed below:

Audit Date	Phase Audited	Date Reported to Study Director	Date Reported to Management
01/28/2010	Procedure	01/28/2010	01/28/2010
01/28/2010	Facilities	01/28/2010	01/28/2010
02/02/2010	Data	02/02/2010	02/02/2010
02/02/2010	Report	02/02/2010	02/02/2010

Chuck Weibel
Quality Assurance Manager

2/3/10
Date

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STUDY PERSONNEL

Testing Facility Management

Daniel L. Prince, Ph.D.
President

2/3/10
Date

Study Director and
Supervisory Personnel

Jozef Mastej
Microbiology Manager

2/3/10
Date

Laboratory Personnel

M. A. Patel
Minal Patel
Microbiologist

2/3/10
Date

Laboratory Personnel

Beverly Marootian
Microbiologist

2/3/10
Date

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STUDY REPORT

STUDY TITLE: EPA Non-food Contact Sanitizer Test on Peraspray

SPONSOR: Enviro Tech Chemical Services, Inc.
500 Winmoore Way
Modesto, CA 95358
Attn: Mike Harvey
Tel #: (209) 581-9578 ext: 104
Fax #: (209) 581-9653
Sponsor #: (1124)
Purchase Order # 161487

TEST FACILITY: Gibraltar Laboratories, Inc.
16 Montesano Road
Fairfield, NJ 07004
Tel #: (973) 227-6882
Fax #: (973) 582-1565

TEST SUBSTANCE IDENTIFICATION

TEST SUBSTANCE NAME: Peraspray; Active Ingredient: Peroxyacetic Acid [PAA 150ppm]

LOT/BATCH NUMBER (S):

GBL # 231523/1 = Lot # 820-9-0814-Lab	Manufacturing Date: 08/14/2009	**>60 days old**
GBL # 231523/2 = Lot # TRNB9-1-56	Manufacturing Date: 10/28/2009	
GBL # 231523/3 = Lot # TRNB9-2-56	Manufacturing Date: 10/28/2009	

DESCRIPTION OF TEST SUBSTANCE: Three white plastic bottles, each with a white plastic screw cap secured with black tape containing a Peraspray. Expiration date is not known. Storage Conditions: The test materials were stored at ambient room temperature at the testing facility. Stability under storage conditions: Stability and purity are the responsibility of the sponsor.

CHEMICAL CHARACTERIZATION: The identity, solubility, stability, strength, purity, and chemical composition were not provided.

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STUDY INITIATION DATE: 12/14/2009
EXPERIMENTAL START DATE: 01/28/2010
EXPERIMENTAL END DATE: 01/30/2010
STUDY COMPLETION DATE: 02/03/2010

STUDY OBJECTIVE: To determine whether or not; The Peraspray kills ≥ 99.9% of *Klebsiella pneumoniae*, ATCC # 4352 and *Staphylococcus aureus*, ATCC # 6538 present on hard surfaces within 5 minutes.

TEST METHOD:
EPA Pesticide Assessment Guidelines Subdivision G: Product Performance 91-2 (j) (p. 55)
EPA Pesticide Assessment Guidelines Subdivision G: Product Performance 91-30 (8), (p. 76)

TEST SYSTEM/STRAINS
Staphylococcus aureus (bacteria), ATCC # 6538; GBL # 171952/8
Klebsiella pneumoniae (bacteria), ATCC # 4352; GBL # 171952/13
Cultures received from American Type Culture Collection, Manassas, Virginia
The purity of the test system was confirmed by streaking onto selective agar and observing for characteristic morphological appearance (i.e., *S. aureus* = small yellow mannitol-fermenting colonies on Mannitol Salt Agar, *K. pneumoniae* = entire, glistening, smooth, grayish-white, and translucent on Nutrient Agar).

STUDY MATERIALS

MEDIA AND REAGENTS
Anatone Broth Lot # L-411
Neutralizer/Recovery Broth (AOAC Letheen Broth containing 0.05% Sodium Thiosulfate) Lot # L-27
Catalase Lot # C-2058, 2074
Trypticase Soy Agar Lot # L-376, A-126
0.01% Triton X-100 solution in Deionized Water, Lot # C-2072
Bovine Calf Serum Lot # 025K84121

EQUIPMENT
Incubator 36 ± 1C
Water bath 20 ± 1C
Calibrated Timer
Calibrated Thermometer
Test Surface (Carriers): 1" x1" glass slides in glass petri dishes matted with two layers of Whatman No.2 filter paper.

STUDY METHOD

PREPARATION OF TEST SUBSTANCE AND METHOD

Test samples were received ready to use (RTU). Using a micropipette, 20 µL of the prepared inoculums containing 5% serum were spread over the entire area of sterile glass slides. The inoculated slides were dried for 35 to 40 minutes at 36 ± 1C then aseptically transferred into individual, sterile, glass jars. Five mL of the test material was added to each jar at 30 second intervals.

PREPARATION OF TEST SYSTEM/STRAINS

Staphylococcus aureus broth culture was grown for 18 to 24 hours at 36 ± 1°C. The culture was vortexed, mixed for 3-4 seconds and allowed to stand 10 minutes at room temperature before continuing. The upper portion of each culture was removed leaving behind any debris or clumps, and was then transferred to a sterile vessel.
Klebsiella pneumoniae was prepared as above.

EXPOSURE CONDITIONS

Contact Time: 1, 3 and 5 minutes
Organic Soil: 5% Bovine Serum in the Inoculum
Test Dilution: Ready To Use [RTU]
Diluent: None
Test Temperature: 20 ± 1C

TEST SYSTEM RECOVERY

After the appropriate contact time, 20 mL of the neutralizer broth was added to each jar and thoroughly mixed by vigorously rotating the jars on a flat surface, for approximately 50 rotations followed by hand agitation. Survivors were enumerated by transferring four-1 mL and four-0.1 mL aliquots from each jar into sterile petri dishes. The plates were poured with TSA and incubated at 36 ± 1C for 48 to 54 hours. Colony-forming units were counted using a dark field Quebec colony counter.

PROTOCOL CHANGES

PROTOCOL AMENDMENTS

None

PROTOCOL DEVIATIONS

One (See attachment # 1 Protocol Deviation)

CONTROLS

PREPARATION OF CONTROLS

Quantitative Control

Two inoculated and dried glass slides, (except disinfection steps), were enumerated. The slides were placed into separate sterile glass jars, 25 mL of neutralizer broth was added to each jar, and the jars were vigorously rotated on a flat surface, for approximately 50 rotations followed by hand agitation. Ten-fold serial dilutions were, made by transferring a 1 mL aliquot from each jar into glass test tubes containing 9 mL of sterile neutralizer broth. Two-1 mL aliquots from each dilution tube were plated into sterile petri dishes. The plates

were poured with TSA and incubated at 36 ± 1C for 48 to 54 hours. Colony-forming units were counted using a dark field Quebec colony counter.

Qualitative Control

Two inoculated and dried glass slides, (except disinfection steps), were placed individually into a sterile glass jars, 25 mL of neutralizer broth were added to each jar, and the jars were vigorously rotated on a flat surface, for approximately 50 rotations followed by hand agitation, then incubated at 36 ± 1C for 48 to 54 hours. Following incubation, the jars were observed for bacterial growth (turbidity).

Negative Control

The control solution (Sterile 0.01% Triton X-100 solution in Deionized Water) was tested in the same manner as the test material. Enumeration of microorganisms was performed by ten-fold serial dilution. A 1 mL aliquot from each jar was transferred into 9 mL of sterile neutralizer broth. Two-1 mL aliquots from each dilution tube were plated into sterile petri dishes. The plates were poured with TSA and incubated at 36 ± 1C for 48 to 54 hours. Colony-forming units were counted using a dark field Quebec colony counter.

Neutralization Challenge

A neutralization confirmation procedure must demonstrate the recovery of a low level (10 to 100 cfu) of the test organism in the neutralizer/subculture tube.

Five mL of the test material was placed into two sterile glass jars. Twenty mL of sterile neutralizer broth were added to each jar and immediately mixed by vigorous rotation of the jars on a flat surface, for approximately 50 rotations. A 24 to 48 hours culture of the test organism was diluted in sterile saline to achieve 100 to 1000 cfu/mL. 0.1 mL of diluted suspension was added to each jar to delivery 10 to 100 cfu per tube. The inoculated jars were incubated for 48 ± 2 hours at 36 ± 1°C and observed for turbidity. Results were recorded as + for growth and 0 for no growth. The neutralization inoculum (e.g. number of bacteria in the 0.1 mL diluted suspension used for inoculation) was conformed by duplicate pour plating 0.1 mL diluted suspension/plate. The plates were poured with TSA and incubated for 48 ± 2 hours at 36 ± 1°C. The colonies on plates were counted and the inoculum was determined. Typical growth in tubes confirms effective neutralization.

STUDY ACCEPTANCE CRITERIA

STUDY REQUIREMENTS

Quantitative Control: At least 1.0 x 10^4 cfu/carrier/organism
Qualitative Control: Carriers produce growth
Negative Controls: At least 1.0 x 10^4 cfu/carrier/organism
Neutralization Challenge: Inoculum counts between 10-100 cfu/jar. Selected jars for neutralization challenge produce growth.

Performance criteria: The results must show a bacterial reduction of at least 99.9% over the parallel control count within 5 minutes.

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DATA ANALYSIS

CALCULATIONS

Basic arithmetic

STATISTICAL ANALYSIS

None

STUDY RETENTION

Data Retention

The final report of this study as well as all raw data accumulated during the study will be kept in the archives of Gibraltar Laboratories, Inc. for a period of at least 10 years, unless notified by sponsor in writing, after which the documents will be returned to the sponsor.

Specimen Retention

After all studies are complete the remaining test material, if any, will be discarded or destroyed in accordance with GBL policy and State and Federal regulations.

STUDY RESULTS

Quantitative Control, Qualitative Control and Negative Controls (Tables 1, 2, 3, 4 and 5): Quantitative control and qualitative control requirements were met. Negative Controls requirements were met. The neutralization challenge requirements were met. The growth was confirmed to be the test organism.

Study Results (Tables 1, 2, 3): For Lot #'s 820-9-0814-Lab, TRNB9-1-56 and TRNB9-2-56 the test substance demonstrate >3 log reduction [>99.9%] after a five minutes contact time against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

STUDY CONCLUSION

Under the conditions of this study "Peraspray" Lot #'s 820-9-0814-Lab, TRNB9-1-56 and TRNB9-2-56, tested ready to-use, in the presence of 5% organic load, EPA Non-Food Contact Sanitizer Test in five minutes contact time against *Staphylococcus aureus* and *Klebsiella pneumoniae.*

REPORT SUBMITTED BY:

Study Director
Jozef Mastej

2/3/2010
Study Completion Date

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Table 1: Test Results Log Reduction, Lot # 820-9-0814-Lab

	Staphylococcus aureus				*Klebsiella pneumoniae*			
	Test sample		0.01% Triton X Control		Test sample		0.01% Triton X Control	
	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}
Inoculum on Carrier After Drying	2.2×10^6	6.34	1.9×10^6	6.28	5.3×10^4	4.72	6.5×10^4	4.81
1 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	2.2×10^4	4.34
3 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	1.5×10^4	4.18
5 Minute	<10	≤ 1	1.4×10^6	6.15	<10	≤ 1	1.5×10^4	4.18
Log Reduction Relative to the Triton X control in Five Minutes	≥ 5.28				≥ 3.81			

TNTC = Too Numerous Too Count, * = average of two carriers; cfu = colony forming units

Table 2: Test Results Log Reduction, Lot # TRNB9-1-56

	Staphylococcus aureus				*Klebsiella pneumoniae*			
	Test sample		0.01% Triton X Control		Test sample		0.01% Triton X Control	
	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}
Inoculum on Carrier After Drying	2.2×10^6	6.34	1.9×10^6	6.28	5.3×10^4	4.72	6.5×10^4	4.81
1 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	2.2×10^4	4.34
3 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	1.5×10^4	4.18
5 Minute	<10	≤ 1	1.4×10^6	6.15	<10	≤ 1	1.5×10^4	4.18
Log Reduction Relative to the Triton X control in Five Minutes	≥ 5.28				≥ 3.81			

TNTC = Too Numerous Too Count, * = average of two carriers; cfu = colony forming units

Table 3: Test Results Log Reduction, Lot # TRNB9-2-56

	Staphylococcus aureus				*Klebsiella pneumoniae*			
	Test sample		0.01% Triton X Control		Test sample		0.01% Triton X Control	
	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}	cfu/carrier*	Log_{10}
Inoculum on Carrier After Drying	2.2×10^6	6.34	1.9×10^6	6.28	5.3×10^4	4.72	6.5×10^4	4.81
1 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	2.2×10^4	4.34
3 Minute	<10	≤ 1	1.5×10^6	6.18	<10	≤ 1	1.5×10^4	4.18
5 Minute	<10	≤ 1	1.4×10^6	6.15	<10	≤ 1	1.5×10^4	4.18
Log Reduction Relative to the Triton X control in Five Minutes	≥ 5.28				≥ 3.81			

TNTC = Too Numerous Too Count, * = average of two carriers; cfu = colony forming units

Table 4: Qualitative Positive Control Results

Test Organisms	Carrier # 1	Carrier # 2
Staphylococcus aureus	(+) Growth	(+) Growth
Klebsiella pneumoniae	(+) Growth	(+) Growth

Table 5: Neutralization Results

		NEUTRALIZATION CONFIRMATION			
Lot #	Test Organism	Date Performed	Inoculum (cfu)*	No. Subculture Vessels	Results
820-9-0814-Lab	*Staphylococcus aureus*	1/28/2010	21	2	(+) Growth
	Klebsiella pneumoniae	1/28/2010	85	2	(+) Growth
TRNB9-1-56	*Staphylococcus aureus*	1/28/2010	21	2	(+) Growth
	Klebsiella pneumoniae	1/28/2010	85	2	(+) Growth
TRNB9-2-56	*Staphylococcus aureus*	1/28/2010	21	2	(+) Growth
	Klebsiella pneumoniae	1/28/2010	85	2	(+) Growth

+ = Typical growth; * = average of two plates; cfu = colony forming units

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Attachment # 1 Protocol Deviation

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PROTOCOL DEVIATION

GBL Study #: GR2635
GBL Protocol #: 3070
Study Title: EPA Non-Food Contact Sanitizer on Peraspray

Sponsor: Enviro Tech Chemical Services, Inc.

Attn.: Mike Harvey
Phone: 209-581-9578 ext. 104
Fax: 209-581-9653

1. In section 10.1 and 10.2 AOAC Letheen Broth with 57.2 units catalase / mL and 0.05% Sodium Thiosulfate (neutralizer/recovery broth)
   Catalase potency = 2,860 units / mg =
   Catalase solution = 200 mg / 100 mL = 5,720 units / mL
   Note: 0.1 mL / 10 mL = 1:100 = 57.2 units catalase / mL.

   Deviation: AOAC Letheen Broth with 286 units catalase / mL and 0.05% Sodium Thiosulfate (neutralizer/recovery broth)
   Catalase potency = 2,860 units / mg =
   Catalase solution = 200 mg / 100 mL = 5,720 units / mL
   Note: 0.5 mL / 10 mL = 1:20 = 286 units catalase / mL

Approved by

Study Director — Date: 1/28/2010

QA Review — Date: 1/28/2010

S:\QA\QA Department\Forms\GLP Forms\Protocol Deviation\EnviroTech GR2635.doc

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