GIBRALTAR LABORATORIES, INC.
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Study Title
AOAC 18th Edition Use-Dilution Test on Peraspray

Product Identity
"Peraspray"

Data Requirement
EPA Pesticide Assessment Guidelines Subdivision G, 1992 Product Performance 91-2 (d) (p.53)

Author
Jozef Mastej
Microbiology Manager

Study Completion Date
02/16/2010

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

Laboratory Project Number (Study File)
GBL Study # GR2636

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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 Chem. Services, Inc

Company Agent MICHAEL HARVEY Date Feb 25, 2010

Pres. Mike 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: EnviroTech Chem Serv Inc 2-25-10
Date

MIKE HARVEY
Study Submitter Name

Reg. MGR.
Study Submitter Title

SPONSOR: Enviro Tech Chemical Services, Inc.

Mike Harvey 2-25-10
Mike Harvey
Study Sponsor Name
Date

STUDY DIRECTOR: Jozef Mastej 2/16/10
Jozef Mastej
Microbiology Manager
Date

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QUALITY ASSURANCE STATEMENT

Study Title: AOAC 18th Edition Use-Dilution Test on Peraspray

Study Number: GR2636

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
01/05/2010	Procedure	01/05/2010	01/05/2010
01/05/2010	Facilities	01/05/2010	01/05/2010
01/29/2010	Data	01/29/2010	01/29/2010
01/29/2010	Report	01/29/2010	01/29/2010

Chuck Weibel
Quality Assurance Manager

2/16/10
Date

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STUDY PERSONNEL

Testing Facility Management	Daniel L. Prince, Ph.D. President	2/16/10 Date
Study Director and Supervisory Personnel	Jozef Mastej Microbiology Manager	2/16/10 Date
Laboratory Personnel	Minal Patel Microbiologist	2/16/10 Date
Laboratory Personnel	Michael Pannullo Microbiologist	2/16/10 Date
Laboratory Personnel	Beverly Marootian Microbiologist	2/16/2010 Date

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STUDY REPORT

STUDY TITLE: AOAC 18th Edition Use-Dilution 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 # 231524/1 = Lot # 820-9-0814-Lab	Manufacturing Date: 08/14/2009	**>60 days old**
GBL # 231524/2 = Lot # TRNB9-1-56	Manufacturing Date: 10/28/2009	
GBL # 231524/3 = 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.

STUDY INITIATION DATE: 12/15/2009
EXPERIMENTAL START DATE: 01/04/2010
EXPERIMENTAL END DATE: 01/29/2010
STUDY COMPLETION DATE: 02/16/2010

STUDY OBJECTIVE: To determine whether the test material kills, in 10 minutes, at least 59 of 60 carriers/lot in each of three lots against the test systems.

TEST METHOD: AOAC 18th Edition
AOAC Official Method 955.14 Testing Disinfectants against *Salmonella entericia* Use-Dilution Method
AOAC Official Method 955.15 Testing Disinfectants against *Staphylococcus aureus* Use-Dilution Method
AOAC Official Method 964.02 Testing Disinfectants against *Pseudomonas aeruginosa* Use-Dilution Method

TEST SYSTEM/STRAINS
Salmonella entericia (bacteria), ATCC # 10708; GBL # 171952/6; Transfer # 11
Staphylococcus aureus (bacteria), ATCC # 6538; GBL # 171952/8; Transfer # 5
Pseudomonas aeruginosa (bacteria), ATCC # 15442; GBL # 171952/12; Transfer # 9
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. entericia* = lactose negative clear colonies on MacConkeys' agar, *S. aureus* = small yellow mannitol-fermenting colonies on Mannitol Salt Agar, *S. entericia* = lactose negative clear colonies on MacConkeys' agar, *Pseudomonas aeruginosa* = smooth, round, fragrant, green pigmentation on Cetrimide Agar).

STUDY MATERIALS

MEDIA AND REAGENTS
Anatone Broth Lot # L-411, K-248
Neutralizer/Recovery Broth (AOAC Letheen Broth containing 0.05% Sodium Thiosulfate) Lot # L-28, 29, 428
Catalase Lot # C-2058
Trypticase Soy Agar Lot # L-375, A-127, 126
Mannitol Salt Agar Lot # L-72
MacConkeys' Agar Lot # L-71
Cetrimide Agar Lot # L-70
Carriers Lot # J-400, A-284
Bovine Calf Serum Lot # 025K84121

EQUIPMENT
Incubator 36 ± 1C
Water bath 20 ± 1C
Calibrated Timer
Calibrated Thermometer

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STUDY METHOD

PREPARATION OF TEST SUBSTANCE AND METHOD

Test samples were received ready to use (RTU). The biologically screened stainless steel penicylinders (8 ± 1 mm o.d. x 6 ± 1 mm i.d. x 10 ± 1 mm length) were soaked overnight (approximately 12 hours) in 1N NaOH. The penicylinders were then rinsed several times with tap water and sterilized at 121C and 15 psi in deionized water. The carriers were soaked for 15 minutes in the 48 hours culture broth containing 5% serum. The carriers were then dried at 36 ± 1C for 40 minutes in a petri dish with sterile filter paper. The product at its use-dilution was distributed into 25 x 150 mm glass disposable test tubes in 10 mL quantities. The samples were brought to 20 ± 1C in a water bath at the same temperature. The contaminated and dried carriers were transferred with a wire hook into the disinfectant for 10 minutes exposure at 30 second staggered intervals.

PREPARATION OF TEST SYSTEM/STRAINS

Salmonella entericia was prepared according to the AOAC 18th Edition Official Method 955.14 Testing Disinfectants against *Salmonella entericia* Use-Dilution Method.
Staphylococcus aureus was prepared according to the AOAC 18th Edition Official Method 955.15 Testing Disinfectants against *Staphylococcus aureus* Use-Dilution Method.
Pseudomonas aeruginosa was prepared according to the AOAC 18th Edition Official Method 964.02 Testing Disinfectants against *Pseudomonas aeruginosa* Use-Dilution Method.

EXPOSURE CONDITIONS

Contact Time: 10 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 10 minutes contact time the carriers were transferred into the recovery medium (AOAC Letheen Broth with 57.2 units catalse / mL and 0.05% Sodium thiosulfate); 10 mL in a 20 x 150 mm test tube for subculture recovery from medicated carrier). The recovery medium tubes (with carriers) were incubated for 48 ± 2 hours at 36 ± 1C in an incubator. Results were recorded as "+" for growth and "0" for no growth. Any positive recovery broth tubes containing carriers were subcultured to appropriate selective/differential agar to confirm the presence of the test system.

PROTOCOL CHANGES

PROTOCOL AMENDMENTS

None

PROTOCOL DEVIATIONS

None

CONTROLS

PREPARATION OF CONTROLS

Quantitative Control

To confirm that at least 1.0 x 10^4 cfu were present on each carrier, three additional contaminated and dried carriers were placed into neutralizer/recovery broth tubes and sonicated for 5 minutes to dislodge the adhering organisms. This was diluted to 10^{-5}, and then two 1.0 mL aliquots from each dilution tube were plated using Trypticase Soy Agar (TSA) and incubated for 24 to 48 hours at 36 ± 1C. The colony counts were extrapolated to cfu per carrier.

Qualitative Control

Two contaminated and dried carriers per organism tested were directly transferred into neutralizer/recovery broth (no disinfection treatment) and incubated for 48 ± 2 hours at 36 ± 1C. Positive growth in each tube validates test system viability.

Sterility Controls

Two sterile Petri dishes were poured with sterile Trypticase Soy Agar (TSA) from each lot of media used in the test and were incubated along with the product. Two sterile vessels containing neutralizer/recovery broth from each lot used in the test were incubated as above. Two times 1.0 mL of bovine serum was added into a sterile vessel containing recovery broth and was incubated as above. Two stainless steel penicylinders were transferred into a sterile vessel containing neutralizer broth and were incubated as above.

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.
At the conclusion of the incubation period, 10 negative carriers for each 60 tubes tested were randomly selected. 24 to 48 hours culture of the test organism were diluted in sterile saline to achieve 100 to 1000 cfu/mL. 0.1 mL, diluted suspension was added to each tube to deliver 10 to 100 cfu per tube. The inoculated test tubes 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 was confirmed (e.g. number of bacteria in the 0.1 mL diluted suspension used for inoculation) 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 were counted on plates to determine inoculum challenge.
Typical growth in tubes confirms effective neutralization.

STUDY ACCEPTANCE CRITERIA

STUDY REQUIREMENTS

Quantitative Control: At least 1.0 x 10^4 cfu/carrier
Qualitative Control: Carriers produce growth
Sterility Controls: Sterile
Neutralization Challenge: Inoculum counts between 10-100 cfu/tube. Selected negative tubes for neutralization challenge produce growth.

Performance criteria: The test substance must kill 59 of 60 carriers/lot/organism within 10 minutes.

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

Study Results (Table 1): For Lot #'s 820-9-0814-Lab, TRNB9-1-56 and TRNB9-2-56 the test substance did inactivate 59 of 60 carriers/lot/test system after a ten minutes contact time against *Salmonella entericia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

STUDY CONCLUSION

Under the conditions of this study "Peraspray" Lot #'s 820-9-0814-Lab, TRNB9-1-56 and TRNB9-2-56, tested eady to-use, in the presence of 5% organic load, did pass the AOAC 18th Edition Use-Dilution Test in ten minutes contact time against *Salmonella entericia, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

REPORT SUBMITTED BY:

Study Director
Jozef Mastej

2/16/10
Study Completion Date

Table 1: Test Results

Test Organism	Lot #	# Positive/# Tested
Salmonella entericia	820-9-0814-Lab	0/60
Staphylococcus aureus		0/60
Pseudomonas aeruginosa		0/60
Salmonella entericia	TRNB9-1-56	0/60
Staphylococcus aureus		0/60
Pseudomonas aeruginosa		0/60
Salmonella entericia	TRNB9-2-56	0/60
Staphylococcus aureus		0/60
Pseudomonas aeruginosa		0/60

Table 2: Carrier Control Results

Test Organism	Date Performed	Result (cfu/carrier)	(cfu/carrier)*	Total average (cfu/carrier)	Qualitative Control
Salmonella entericia	1/05/2010	3.9×10^4	2.8×10^4	2.2×10^4	+, +
		2.1×10^4			
		2.3×10^4			
		2.6×10^4	2.2×10^4		+, +
		1.8×10^4			
		2.1×10^4			
		1.3×10^4	1.6×10^4		+, +
		1.8×10^4			
		1.8×10^4			
Salmonella entericia	1/05/2010	1.2×10^4	1.4×10^4	3.8×10^4	+, +
		1.7×10^4			
		1.3×10^4			
		3.7×10^4	5.1×10^4		+, +
		6.4×10^4			
		5.1×10^4			
		4.5×10^4	4.8×10^4		+, +
		6.0×10^4			
		4.0×10^4			

Legend: + = Typical growth; * = average of three carriers; cfu = colony forming units

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Table 2: Carrier Control Results

Test Organism	Date Performed	Result (cfu/carrier)	(cfu/carrier)*	Total average (cfu/carrier)	Qualitative Control
Salmonella entericia	1/05/2010	3.4×10^4	4.0×10^4	3.1×10^4	+, +
		3.5×10^4			
		5.2×10^4			
		3.4×10^4	3.9×10^4		+, +
		4.8×10^4			
		3.4×10^4			
		1.3×10^4	1.3×10^4		+, +
		1.2×10^4			
		1.5×10^4			
Staphylococcus aureus	1/21/2010	9.4×10^5	9.6×10^5	1.1×10^6	+, +
		9.7×10^5			
		9.8×10^5			
		1.1×10^6	1.2×10^6		+, +
		1.2×10^6			
		1.2×10^6			
		1.2×10^6	1.2×10^6		+, +
		1.2×10^6			
		1.2×10^6			
Staphylococcus aureus	1/21/2010	1.1×10^6	1.1×10^6	1.2×10^6	+, +
		1.0×10^6			
		1.2×10^6			
		1.4×10^6	1.3×10^6		+, +
		1.2×10^6			
		1.3×10^6			
		1.1×10^6	1.1×10^6		+, +
		1.1×10^6			
		1.2×10^6			
Staphylococcus aureus	1/21/2010	1.3×10^6	1.6×10^6	2.0×10^6	+, +
		1.9×10^6			
		1.5×10^6			
		3.6×10^6	2.6×10^6		+, +
		2.2×10^6			
		2.0×10^6			
		1.8×10^6	1.7×10^6		+, +
		1.7×10^6			
		1.5×10^6			

Legend: + = Typical growth; * = average of three carriers; cfu = colony forming units

Table 2: Carrier Control Results

Test Organism	Date Performed	Result (cfu/carrier)	(cfu/carrier)*	Total average (cfu/carrier)	Qualitative Control
Pseudomonas aeruginosa	1/25/2010	2.5×10^6	2.1×10^6	1.8×10^6	+, +
		1.9×10^6			
		1.8×10^6			
		1.3×10^6	1.4×10^6		+, +
		1.9×10^6			
		1.0×10^6			
		2.1×10^6	1.9×10^6		+, +
		2.2×10^6			
		1.3×10^6			
Pseudomonas aeruginosa	1/25/2010	1.5×10^6	1.3×10^6	1.6×10^6	+, +
		1.1×10^6			
		1.2×10^6			
		1.6×10^6	1.2×10^6		+, +
		1.0×10^6			
		1.1×10^6			
		2.0×10^6	2.2×10^6		+, +
		2.9×10^6			
		1.8×10^6			
Pseudomonas aeruginosa	1/25/2010	2.1×10^6	2.3×10^6	1.5×10^6	+, +
		2.4×10^6			
		2.5×10^6			
		5.0×10^5	6.1×10^5		+, +
		8.8×10^5			
		4.5×10^5			
		1.5×10^6	1.6×10^6		+, +
		1.8×10^6			
		1.4×10^6			

Legend: + = Typical growth; * = average of three carriers; cfu = colony forming units

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Table 3: Neutralization Results

Lot #	Test Organism	NEUTRALIZATION CONFIRMATION			
		Date Performed	Inoculum (cfu)	No. Subculture Tubes Tested	Results
820-9-0814-Lab	*Salmonella entericia*	1/07/2010	35	10	Growth
	Staphylococcus aureus	1/25/2010	44	10	Growth
	Pseudomonas aeruginosa	1/27/2010	62	10	Growth
TRNB9-1-56	*Salmonella entericia*	1/07/2010	35	10	Growth
	Staphylococcus aureus	1/25/2010	44	10	Growth
	Pseudomonas aeruginosa	1/27/2010	62	10	Growth
TRNB9-2-56	*Salmonella entericia*	1/07/2010	35	10	Growth
	Staphylococcus aureus	1/25/2010	44	10	Growth
	Pseudomonas aeruginosa	1/27/2010	62	10	Growth

Table 4: Sterility Check Results

Media	Lot #	Results
Trypticase Soy Agar (TSA)	L-375, A-127, 126	Sterile, Sterile, Sterile
Neutralizer/Recovery Broth	L-28, 29, 428	Sterile, Sterile, Sterile
Carriers	J-400, A-284	Sterile, Sterile
Bovine Serum	025K8412	Sterile