GIBRALTAR LABORATORIES, INC.
MICROBIOLOGY
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Study Title
EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

Product Identity
"Reflexx"

Data Requirement
EPA DIS/TSS-4 of January 30, 1979

Author
Daniel L. Prince, Ph.D.
President

Study Completion Date
02/08/2008

Testing Facility
Gibraltar Laboratories, Inc.
122 Fairfield Road
Fairfield, NJ 07004

Laboratory Project Number (Study File)
GBL Study # GR 2328

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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. Serv. Inc

Company Agent _____ Date 2-15-08

Pres. Michael Harvey

Title Signature

Note: Applicants for permanent or temporary tolerances should note that it is OPP Policy that no permanent or temporary tolerance petition or request for an emergency exemption, that incorporates an analytical method, can be approved unless the applicant waives all claims of confidentiality for the analytical method. These analytical methods are published in the FDA Analytical Methods Manual, and therefore cannot be claimed as confidential. OPP implements this policy by returning submitted analytical methods (for which confidentiality claims have been made) to the submitter, to obtain the confidentiality waiver before they can be processed.

*Gibraltar Laboratories, Inc. is not aware of what, if any, data will be classified as confidential. Accordingly, this page is left blank for the sponsor to complete.

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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: Enviro Tech Chem Inc 2-15-08
Date

Michael Harvey
Study Submitter Name

Pres
Study Submitter Title

SPONSOR: Enviro Tech Chemical Services, Inc.

Michael Harvey 2-15-08
Michael Harvey Date
Study Contact Name

STUDY DIRECTOR: Jozef Mastej 2/8/08
Jozef Mastej Date
Disinfectant Testing Manager

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QUALITY ASSURANCE STATEMENT

Study Title: EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

Study Number: GR2328

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
01/09/2008	Procedure	01/09/2008	01/09/2008
01/09/2008	Facilities	01/09/2008	01/09/2008
01/12/2008	Data	01/12/2008	01/12/2008
02/04/2008	Report	02/04/2008	02/04/2008

Chuck Weibel
Quality Assurance Manager

2/8/08
Date

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STUDY PERSONNEL

Testing Facility Management	Daniel L. Prince, Ph.D. President	2/8/08 Date
Study Director and Supervisory Personnel	Jozef Mastej Disinfectant Testing Manager	2/8/08 Date
Laboratory Personnel	Minal Patel Microbiologist	2/8/08 Date
Laboratory Personnel	Neil Eppinger Microbiologist	2/8/08 Date
Laboratory Personnel	Michael Pannullo Microbiologist	2/8/08 Date

STUDY REPORT

STUDY TITLE: EPA Food Contact Sanitizer Test For Previously Cleaned Food-Contact Surfaces (AOAC Germicidal and Detergent Sanitizing Action of Disinfectants)

SPONSOR: Enviro Tech Chemical Services, Inc.
500 Winmoore Way
Modesto, CA 95358
Attn: Michael Harvey
Tel #: 209/581-9576
Fax #: 209/581-9653
Sponsor #: (1124)
Purchase Order # N/A

TEST FACILITY: Gibraltar Laboratories, Inc.
122 Fairfield Road
Fairfield, NJ 07004
Tel #: 973/227-6882
Fax #: 973/227-0812

TEST SUBSTANCE IDENTIFICATION

TEST SUBSTANCE NAME: "Reflexx"; an equilibrium mixture of Hydrogen Peroxide/Peroxyacetic Acid; Concentration on file at Enviro Tech Chemical Services.

LOT/BATCH NUMBER (S): Expiration date not known.
GBL # 198736/1 = Lot # 037-L070822; Manufacturing Date: August 22, 2007
GBL # 198736/2 = Lot # 037-L070910-1; Manufacturing Date: September 10, 2007
GBL # 198736/3 = Lot # 037-L070912; Manufacturing Date: September 12, 2007

DESCRIPTION OF TEST SUBSTANCE: Three clear glass bottles, each with a white plastic screw cap secured with black tape containing Reflexx. 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: 01/02/2007
EXPERIMENTAL START DATE: 01/09/2008
EXPERIMENTAL END DATE: 01/12/2008
STUDY COMPLETION DATE: 02/08/2008

STUDY OBJECTIVE: To determine whether or not "Reflexx", kills 99.999% of *Staphylococcus aureus* and *Escherichia coli* within 30 seconds in a suspension test.

TEST METHOD: AOAC 18th Edition Chapter 6.3.03; AOAC Official Method 960.09 "Germicidal and Detergent Sanitizing Action of Disinfectants"

TEST SYSTEM/STRAINS:
- *Staphylococcus aureus* (bacteria), GBL # 171952/8, ATCC # 6538
- *Escherichia coli* (bacteria), GBL # 171952/10, ATCC # 11229

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, *E.coli* = brick-red; may have surrounding zone of precipitated bile on MacConkey Agar).

STUDY MATERIALS

MEDIA AND REAGENTS

Nutrient Agar A Lot # K-435
Nutrient Agar B Lot # K-436
Phosphate Buffer Stock Solution, Lot # J-279
Phosphate Buffer Dilution Water, Lot # K-437
Tryptone Glucose Extract Agar Lot # K-433, 434
200 ppm AOAC Hard Water, Lot # C-1871, C-1873
0.05% Sodium thiosulfate Lot # C-1847
Catalase solution Lot # C-1872
Neutralizer Broth (Phosphate Buffer Dilution Water containing 57.2 units catalase / mL and 0.05% Sodium thiosulfate)

EQUIPMENT

Incubator 37 ± 1C
Water-bath 25 ± 0.2C

STUDY METHOD

PREPARATION OF TEST SUBSTANCE AND METHOD

One mL of the test substance was added to 895 mL of 200 ppm AOAC hard water (volume to volume) to equal 64.7 ppm(PAA) and 223.2 ppm(H_2O_2) active. 99 mL of water to be used in the test, containing bactericide at the concentration to be tested, was measured into sterile, 250 mL wide-mouth Erlenmeyer flasks and placed in a constant temperature bath until it reached 25 ± 0.2C, for ≥20 min. Duplicate flasks were prepared for each germicide to be tested. A similar flask was also prepared containing

99 mL sterile phosphate buffer dilution H_2O, as "initial numbers" control. One mL of culture suspension was added to each test flask as follows: The flask was whirled, stopping just before suspension was added, creating enough residual motion of liquid to prevent pooling of suspension at the point of contact with the test water. The suspension was added midway between center and edge of surface with tip of pipet slightly immersed in the test solution. Care was taken to avoid touching the pipet to the neck or side of flask during the addition. One mL portions of this exposed culture were added to neutralizer blank exactly 30 and 60 seconds after the addition of the suspension and mixed well immediately after transfer. For test samples, the following dilution procedure was followed: 1 mL-exposed culture was transferred into 9 mL neutralizer broth and vortexes to dislodge adhering organisms. 1 mL and 0.1 mL were plated in **quadruplicate** and poured with Tryptone Glucose Extract Agar (TGEA). Plates were incubated for 48 hours at 37 ± 1C. The colony forming units were counted using Quebec colony counter.

PREPARATION OF TEST SYSTEM/STRAINS

Staphylococcus aureus and *Escherichia coli* were prepared according to the AOAC 18th Edition, section 960.09D.

EXPOSURE CONDITIONS

Contact Time: 30 and 60 seconds
Organic Soil: none
Test Concentration: 1ounce/ 7gallons (1:896)
Test Dilution: 1 mL test substance + 895 mL diluent
Diluent: sterile 200 ppm AOAC hard water
Test Temperature: 25 ± 0.2C

TEST SYSTEM RECOVERY

For incubation, the organisms will be incubated at 37 ± 1C and not 35C, as this is an acceptable temperature range for the mesophilic test system organisms for 48 ± 8 hours or longer according to the best judgment of the bacteriologist.

PROTOCOL CHANGES

PROTOCOL AMENDMENTS

None

PROTOCOL DEVIATIONS

None

CONTROLS

PREPARATION OF CONTROLS

Number Controls

The "number controls" were performed by adding 1 mL of the appropriate test system organism to **duplicate** 250 mL wide-mouth Erlenmeyer flasks containing 99 mL of the sterile phosphate buffer dilution water. The number of bacteria present in the Erlenmeyer flasks was determined after ≤ 30

seconds. Ten-fold serial dilutions were made into 9 mL neutralizer broth; 1 mL pour plates in **quadruplicate** were performed using TGEA. Plates were incubated for 48 hours at 37 ± 1C. The colony forming units were counted using Quebec colony counter.

Neutralization Effectiveness Control

Neutralizer effectiveness was verified for each organism type and each lot germicide tested. 1.0 mL of the bactericide at the concentration to be tested was transferred into each sterile test tube containing 8 mL of neutralizer broth. 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at 37 ± 1C. The colony forming units were counted using Quebec colony counter.

Neutralization System Toxicity Control

Neutralizer system toxicity was verified for each organism type to assure that the neutralizing system is not toxic to the bacterial cells (If toxicity is present, the organism will not grow). 9 mL of neutralizer broth was tested into sterile test tube. 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at 37 ± 1C. The colony forming units were counted using Quebec colony counter.

Inoculum Counts

To verify the count of the inoculum is 10 to 100 cfu/mL, 1 mL of 100 to 1000 cfu/mL of the test organism was inoculated into each test tube containing 9 mL of Phosphate Buffer Dilution Water within 30 seconds and vortexed. 1 mL pour plates in **duplicate** were performed using TGEA. Plates were incubated for 48 hours at 37 ± 1C. The colony forming units were counted using Quebec colony counter.

Sterility Controls of Neutralizer Broth, AOAC Hard Water and Phosphate Buffer Dilution Water:

2 x 1.0 mL of Neutralizer broth, AOAC hard water was individually plated into sterile petri dishes and the plates were poured with TGEA. The plates were incubated at 37 ± 1C for 48 ± 8 hours. The colony forming units were counted using Quebec colony counter.

STUDY ACCEPTANCE CRITERIA

STUDY REQUIREMENTS

1) Number Controls: counts between 75 and 125 x 10^6
   Neutralizer Effectiveness: counts between 10 and 100 cfu/mL
   Neutralizer Toxicity: counts between 10 and 100 cfu/mL
   Inoclum Counts: counts between 10 and 100 cfu/mL
   Sterility Controls: sterile
2) Performance criteria: the test substance must demonstrate a 99.999% kill within 30 seconds.

DATA ANALYSIS

CALCULATIONS

Basic arithmetic and transformation by Log_{10}

STATISTICAL ANALYSIS

None

STUDY RETENTION

Data Retention

The test findings reflected in this experiment will be kept on file for a period of at least five years in the Gibraltar Laboratories archives. Specific records to be maintained include a copy of this report, all raw data, sample information as provided by sponsor, and the findings of the QAU.

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

Control and Neutralization Results (Tables 5 and 6): Number controls, neutralizer effectiveness, neutralizer toxicity, inoculum counts and sterility control requirements were met.

Study Results (Table 1-4):

- Table 1-2 The test substance inactivated >99.9% of the *Staphylococcus aureus* within 30 seconds at a 64.7 ppm(PAA) and 223.2 ppm(H_2O_2) concentration at 25C.
- Table 3-4 The test substance inactivated >99.999% of the *Escherichia coli* within 30 seconds at a 64.7 ppm(PAA) and 223.2 ppm(H_2O_2) concentration at 25C.

STUDY CONCLUSION

Reflexx, Lot # 037-L070822, Lot # 037-L070910-1 and Lot # 037-L070912 prepared in 200 ppm AOAC hard water inactivated > 99.999% of *Escherichia coli* and >99.9% of *Staphylococcus aureus* when tested at 1ounce / 7gallons 64.7 ppm(PAA) and 223.2 ppm(H_2O_2) at 25C within 30 seconds contact time.

REPORT SUBMITTED BY:

Jozef Mastej
Study Director

2/8/08
Study Completion Date

Table 1: Raw Data Results for *Staphylococcus aureus*

Test Substance	Concentration in ppm (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Number Controls (10^{-6}) (Microbes Initially Present)	
				10^{-1}	10^{-2}	Flask A	Flask B
"Reflexx" Lot # 037-L070822	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	>300, >300, >300, >300	266, 289, 290, 259	1.2 x 10^8 1.1 x 10^8 1.0 x 10^8 1.0 x 10^8	1.2 x 10^8 1.3 x 10^8 1.2 x 10^8 1.3 x 10^8
			Flask B	>300, >300, >300, >300	243, 269, 193, 181		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-L070910-1	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	>300, >300, >300, >300	228, 223, 227, 195		
			Flask B	>300, >300, >300, >300	281, 296, 235, 202		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-L070912	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	>300, >300, >300, >300	183, 165, 193, 221		
			Flask B	>300, >300, >300, >300	236, 211, 151, 198		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
						Avg. of Flask A and B 1.2 x 10^8 = 8.08 Log	

Table 2: Calculated Results for *Staphylococcus aureus* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log_{10} Reduction

Test Substance / Lot #	Exposure Time	Concentration in ppm (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log_{10})	Log_{10} Reduction	Percent Reduction
"Reflexx" Lot # 037-L070822	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	2.5 x 10^4	1.2 x 10^8	8.08	3.68	>99.9%
"Reflexx" Lot # 037-L070910-1	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	2.3 x 10^4	1.2 x 10^8	8.08	3.72	>99.9%
"Reflexx" Lot # 037-L070912	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	2.0 x 10^4	1.2 x 10^8	8.08	3.78	>99.9%

Table 3: Raw Data Results for *Escherichia coli*

Test Substance	Concentration in ppm (v/v)	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Number Controls (10^{-6}) (Microbes Initially Present)	
				10^{-1}	10^{-2}	Flask A	Flask B
"Reflexx" Lot # 037-L070822	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	0,0,0,0	0,0,0,0	1.1×10^8 1.1×10^8 1.2×10^8 1.2×10^8	1.1×10^8 1.2×10^8 1.2×10^8 1.1×10^8
			Flask B	0,0,0,0	0,0,0,0		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-L070910-1	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
"Reflexx" Lot # 037-L070912	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	30 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
		60 seconds	Flask A	0,0,0,0	0,0,0,0		
			Flask B	0,0,0,0	0,0,0,0		
						Avg. of Flask A and B $1.2 \times 10^8 = 8.08$ Log	

Table 4: Calculated Results for *Escherichia coli* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log_{10} Reduction

Test Substance / Lot #	Exposure Time	Concentration in ppm (v/v)	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log_{10})	Log_{10} Reduction	Percent Reduction
"Reflexx" Lot # 037-L070822	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	<10	1.2×10^8	8.08	≥7.08	>99.999%
"Reflexx" Lot # 037-L070910-1	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	<10	1.2×10^8	8.08	≥7.08	>99.999%
"Reflexx" Lot # 037-L070912	30 seconds	64.7 ppm(PAA) 223.2 ppm(H_2O_2)	<10	1.2×10^8	8.08	≥7.08	>99.999%

Table 5: Neutralization Effectiveness and Neutralization System Toxicity Control Results

Test Substance and Test	*Staphylococcus aureus*			*Escherichia coli*		
	Test Tube A (cfu/mL)	**Test Tube B (cfu/mL)**	**Inoculum (cfu/mL)**	**Test Tube A (cfu/mL)**	**Test Tube B (cfu/mL)**	**Inoculum (cfu/mL)**
Neutralization Effectiveness Control "Reflexx" Lot # 037-L070822	88	95	94	31	25	36
Neutralization Effectiveness Control "Reflexx" Lot # 037-L070910-1	96	93		28	26	
Neutralization Effectiveness Control "Reflexx" Lot # 037-L070912	91	89		25	28	
Neutralization System Toxicity Control (Neutralizer Broth)	93	95		33	31	

Table 6: Sterility Control Results

Reagents and Lot #'s	Result
AOAC Hard Water Lot # C-1871, C-1873	Sterile, Sterile
Phosphate Buffer Dilution Water Lot # K-437	Sterile
Tryptone Glucose Extract Agar (TGEA) Lot # K-433, K-434	Sterile, Sterile
Neutralizer Broth (Phosphate Buffer Dilution Water containing 57.2 units catalase / mL and 0.05% Sodium thiosulfate) Lot # K-437	Sterile