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REPORT No.

G-38559

8/9/2001

EDUCATION *Ph.D. Supervised*
EXPERIENCE *Serving since 1970*
EXTRAORDINARY *Upstream QA™*
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LABORATORY REPORT

EXCELLENCE = GIBRALTAR

Study Title

Food Contact Sanitizer Test on BioSide HS 5%
Against *Salmonella choleraesuis*

Product Identity

BioSide HS 5%

Data Requirement

EPA Pesticide Assessment Guidelines Subdivision G, 1982, 91-2.

Author

Daniel L. Prince, Ph.D.
President

Study Completion Date

July 14, 2001

Testing Facility

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

Laboratory Project Number (Study File)

GBL Study # GR 1722

Page 1 of 11

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Page 2 of 11

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- No claim of confidentiality under FIFRA 10(d)(1)(A),(B), or (C)

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 M HARVEY Date 8-17-01
Pres. Mike Harvey
 Title Signature

- Claim of confidentiality under FIFRA 10(d)(1)(A),(B), or (C)

Statement of Data Confidentiality Claims

Information claimed confidential on the basis of its falling within the scope of FIFRA 10(d)(1)(A),(B), or (C) has been removed to a confidential appendix, and is cited by cross-reference number in the body of the study.
 Company _____
 Company Agent _____ Date _____
 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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Page 3 of 11

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GLP Compliance Statement

This study was conducted in accordance with the principles of Good Laboratory Practices (GLP) as promulgated by the following regulatory agency:

40 CFR Ch.1 (7-1-99 edition) Part 160 Good Laboratory Practice Standards (EPA - FIFRA Pesticides).

Study No.: GR 1722

Protocol No.: 2290

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.

Jozef Mastej
 Study Director

8/13/01
 Date

MIKE HARVEY (EnviroTech)
 Study Sponsor *Mike Harvey*

8-17-01
 Date

Mike Harvey
 Study Submitter

8-17-01
 Date

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Page 4 of 11

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Quality Assurance Statement

Study Number: GR 1722

Protocol Number: 2290

Sponsor Name: Enviro Tech Chemical Services Inc.

Type of Study: Food Contact Sanitizer Test

Audit Dates

Audits were conducted on the following dates.

Procedure 7/12/01
Facilities 7/12/01
Data 8/1/01
Report 8/1/01

Findings

Any findings were communicated to the individuals listed below and to the appropriate personnel involved with the study in a timely fashion.

Findings Reported to

8/1/01
Jozef Mastej
Study Director

8/1/01
J. C. Knutsen, Ph.D.
Management

Amy Huhn
Quality Assurance

8/14/01
Date

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REPORT No.
 G-38559
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Page 5 of 11

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Study Personnel

Testing Facility Management:

Daniel L. Prince, Ph.D.
 President

Supervisory Personnel

J. C. Knutsen, Ph.D.
 Scientific Director

**Study Director and
 Laboratory Personnel**

Jozef Mastej
 Study Director/Microbiologist

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REPORT No.

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Page 6 of 11

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Contents

Title Page 1

Confidentiality Statement 2

GLP Compliance Statement 3

Quality Assurance Statement 4

Study Personnel 5

Table of Contents 6

Purpose, Test System, Method 7

Additional Information 8

Results

Conclusion 11

Table 1 to 4 11

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Page 7 of 11

REPORT No.

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- 1 **Purpose**
 To determine whether or not the test materials kills 99.999% of *Salmonella choleraesuis* within 30 seconds in a suspension test.
- 2 **Test System**
Salmonella choleraesuis; GBL-NJ Login # 106771/7; ATCC # 10708
- 3 **Disinfectant Tested:** BioSide HS 5%
- 4 **Test Conditions**
 - 4.1 Contact Time: 30 seconds and 60 seconds
 - 4.2 Organic Soil: none
 - 4.3 Test Concentration: 1 ounce/5 gallons (1:640)
 - 4.4 Test Dilution: 1 mL Test Material + 639 mL Diluent
 - 4.5 Diluent: sterile 400 ppm AOAC hard water
 - 4.6 Test Temperature: 20 ± 0.2C.
- 5 **Preparation of Culture:** Organisms were prepared according to the AOAC.
- 6 **Method**
 99 mL of the diluted test material prepared as in section 4.4 was aliquoted into each of two wide-mouth erlenmeyer flasks. The flasks were allowed to equilibrate for ≥ 20 minutes in a 20± 0.2C water bath. In parallel, two flasks were prepared as numbers controls where in sterile phosphate buffer dilution water was substituted for the 99 mL of test material. The efficacy assay was performed by adding 1 mL of the appropriate test system organism to the 99 mL flask as per AOAC. The number of bacteria present in the erlenmeyer flasks was determined after the 30 seconds and 60 seconds contact time. Ten-fold serial dilutions were made into 9 mL GBL STAT Broth (GBL STAT Broth = Trypticase Soy Broth containing 4% Tween 20 and 0.5% Azolectin) and pour plates were performed in quadruplicate using Tryptone Glucose Extract Agar (TGEA) containing neutralizer. Incubation was for 48 hours at 37 ± 1C. The colony forming units were counted using a Quebec colony counter.

 The numbers control was performed by adding 1 mL of the appropriate test system organism to duplicate wide-mouth erlenmeyer flasks containing 99 mL of the sterile phosphate buffer dilution water as per AOAC. The number of bacteria present in the erlenmeyer flasks was determined after ≤ 30 seconds. Ten-fold serial dilutions were made into 9 mL GBL STAT Broth. Pour plates were performed in quadruplicate were performed using TGEA. Incubation was for 48 hours at 37 ± 1C. The colony forming units were counted using a Quebec colony counter.

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Page 8 of 11

REPORT No.

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7. **Sterility Controls**
 For neutralizer broth, AOAC hard water and Phosphate buffer dilution water, 1.0 mL of each was individually plated into sterile petri dishes and the plates were poured with TGEA. The plates were incubated at 37 ± 1 C for 48 ± 8 hours. The colony forming units were counted using Quebec colony counter.
8. **Confirmation of Surviving Organisms**
 After counting the plates, colonies of the surviving organisms, if any, were selected and subcultured onto respective agar plates and incubated at 37 ± 1 C for 24 to 48 hours to confirm the typical growth.
9. **Media and Reagents**
- 9.1 Nutrient Agar A, Lot # F-223
 - 9.2 Nutrient Agar B, Lot # G-2
 - 9.3 Phosphate Buffer Stock Solution, Lot # F-211
 - 9.4 Phosphate Buffer Dilution Water, Lot # F-226
 - 9.5 GBL STAT Broth, (Trypticase Soy Broth containing 4.0% Tween 20 and 0.5% Azolectin), Lot # F-196
 - 9.6 AOAC Hard Water, Lot # C-801
 - 9.7 Tryptone Glucose Extract Agar (TGEA), Lot # G-119
10. **Additional Information**
- 10.1 **Purchase Order #: NA**
Sponsor #: (1124)
 Enviro Tech Chemical Services, Inc.
 213 Primo Way
 Modesto, CA 95358
Attn: Mike Harvey
Tel #: 209/581-9576
Fax #: 209/581-9653
GBL Reference #: 27-527-128
GBL-NJ Sample #: 28545/1-2.262
Study Initiation: 06/29/01
Date Received: 06/19/01
Date Tested: 07/12/01
Date Completed: 07/14/01
Protocol No.: 2290

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Page 9 of 11

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10.2 Description of Test Material

Two plastic bottles with white plastic screw caps each containing BioSide HS 5%.
 28545/1 = Lot # 01206 Manufacturing Date: Dec. 6, 2000
 28545/2 = Lot # 10102 Manufacturing Date: Jan. 2, 2001

10.3 Statistical Method: None. Basic arithmetic was used.

10.4 Records to be Maintained: 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.

10.5 Other Remarks Pertaining To The Test Material

10.5.1 Chemical Nature and Concentration: Hydrogen Peroxide/Peroxyacetic Acid; Concentration on file at Enviro Tech Chemical Services

10.5.2 Expiration Date: Not known by Gibraltar.

10.5.3 Storage Conditions: The test materials were stored at ambient room temperature at the testing facility.

10.5.4 Stability under above Conditions: Stability and purity are the responsibility of the sponsor.

10.5.5 Sample 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.

10.6 Archive Location: Gibraltar Laboratories, 122 Fairfield Road, Room 203A, Fairfield, New Jersey 07004-2405; or Guarantee Records Management, 215 Coles Street, Jersey City, New Jersey 07307.

10.7 Changes from the Approved Protocol: None.

10.8 Circumstances

No circumstances arose during the course of this study which compromised the quality or integrity of the data reported herein.

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Page 10 of 11

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Table 1: Raw Data Results for *Salmonella choleraesuis*

Test Substance	Concentration in ppm	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Microbes Initially Present (Control)	
				Test 0.1 mL (10 ⁻²) in neutralizer	Test 1.0 mL (10 ⁻¹) in neutralizer	No. Control 1.0 mL of 10 ⁻⁶	
				Flask A	Flask B	Flask A	Flask B
BioSide HS 5% Lot # 01206	78 ppm	30 seconds	Flask A	0,0,0,0	0,0,0,0	88,91, 65,74	98,89, 81,84
			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		

Table 2: Calculated Results for *Salmonella choleraesuis* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log₁₀ Reduction

Test Substance	Exposure Time	Concentration in ppm	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log ₁₀)	Log ₁₀ Reduction	Percent Reduction
BioSide HS 5% Lot # 01206	30 seconds	78 ppm	<10	8.4 x 10 ⁷	7.92	≥ 6.92	≥ 99.999%

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Page 11 of 11

REPORT No.
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Table 3: Raw Data Results for *Salmonella choleraesuis*

Test Substance	Concentration in ppm	Exposure Time	Flask	Plate Counts (CFU/plate)			
				Number Surviving		Microbes Initially Present (Control)	
				Test 0.1 mL (10 ⁻³) in neutralizer	Test 2.0 mL (10 ⁻¹) in neutralizer	No. Control 1.0 mL of 10 ⁻⁶	
				Flask A	Flask B	Flask A	Flask B
BioSide HS 5% Lot # 10102	78 ppm	30 seconds	Flask A	0,0,0,0	0,0,0,0	88,91, 65,74	98,89, 81,84
			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		

Table 4: Calculated Results for *Salmonella choleraesuis* (cfu/mL) by Lot, Exposure, and corresponding Percent and Log₁₀ Reduction

Test Substance	Exposure Time	Concentration in ppm	Average Number Surviving (cfu/mL)	Microbes Initially Present (cfu/mL)	Microbes Initially Present (Log ₁₀)	Log ₁₀ Reduction	Percent Reduction
BioSide HS 5% Lot # 10102	30 seconds	78 ppm	<10	8.4 x 10 ⁷	7.92	≥ 6.92	≥ 99.999%

Conclusion

BioSide HS 5%, Lot # 01206 and 10102, killed 99.999% of *Salmonella choleraesuis* within 30 seconds when diluted 1 ounce/5 gallons (1:640) in 400 ppm AOAC hard water.

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