



EDGEWOOD

CHEMICAL BIOLOGICAL CENTER

U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND

ECBC-TR-521

**BIOLOGICAL-WARFARE AGENT DECONTAMINATION EFFICACY TESTING:
LARGE-SCALE CHAMBER
mVHP® DECONTAMINATION SYSTEM EVALUATION
FOR BIOLOGICAL CONTAMINATION**

Teri A. Lalain
Mark D. Brickhouse
Jerry W. Pfarr

RESEARCH AND TECHNOLOGY DIRECTORATE

Stanley C. Gater
James P. Hendershot

ENGINEERING DIRECTORATE

Brent A. Mantooth



GEO-CENTERS, INC. - GUNPOWDER BRANCH

David C. Stark



EAI CORPORATION
Abingdon, MD 21009

August 2007

Approved for public release;
distribution is unlimited.



20070924089

ABERDEEN PROVING GROUND, MD 21010-5424

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188													
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.																	
1. REPORT DATE (DD-MM-YYYY) XX-08-2007		2. REPORT TYPE Final		3. DATES COVERED (From - To) Oct 2005 – Mar 2006													
4. TITLE AND SUBTITLE Biological-Warfare Agent Decontamination Efficacy Testing: Large-Scale Chamber mVHP® Decontamination System Evaluation for Biological Contamination				5a. CONTRACT NUMBER W9115R-04-C-0024													
				5b. GRANT NUMBER													
				5c. PROGRAM ELEMENT NUMBER													
6. AUTHOR(S) Lalain, Teri A.; Brickhouse, Mark D.; Pfarr, Jerry W.; Gater, Stanley C.; Hendershot, James P. (ECBC); Mantooth, Brent A. (GEO-CENTERS, INC.); and Stark, David C. (EAI Corporation)				5d. PROJECT NUMBER None													
				5e. TASK NUMBER													
				5f. WORK UNIT NUMBER													
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) DIR, ECBC, ATTN: AMSRD-ECB-RT//AMSRD-ECB-ENT, APG, MD 21010-5424 GEO-CENTERS, INC., Gunpowder Branch, P.O. Box 68, APG, MD 21010-0068 EAI Corporation, 1308 Continental Drive, Suite J, Abingdon, MD 21009				8. PERFORMING ORGANIZATION REPORT NUMBER ECBC-TR-521													
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)													
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)													
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.																	
13. SUPPLEMENTARY NOTES																	
14. ABSTRACT: The STERIS Vaporous Hydrogen Peroxide (VHP®) technology has been used for more than a decade to sterilize pharmaceutical processing equipment and clean rooms. Through a joint partnership, the U.S. Army Edgewood Chemical Biological Center (ECBC) and STERIS Corporation, Inc., a subsidiary of Strategic Technology Enterprises (STE), began the process to co-develop a modified VHP (mVHP) capable of biological and chemical decontamination. Significant improvements have been made through a series of laboratory, chamber-scale, and large-scale efforts. The primary objective of this test was to determine the mVHP system's ability to decontaminate biological-warfare agent contamination on operationally relevant materials. The decontamination efficacy was compared to the Key Performance Parameters (KPPs) stated in the Operational Requirements Document (ORD) for Joint Platform Interior Decontamination (JPID). The decontamination efficacy was compared also to the KPPs stated in the ORD for Joint Service Sensitive Equipment Decontamination (JSSED). The tests were performed between October 2005 and March 2006 in the Engineering Directorate large-scale chambers at ECBC, APG, MD. The results for the biological agent and surrogate studies are presented in this report.																	
15. SUBJECT TERMS <table border="0" style="width: 100%;"> <tr> <td>Vaporized Hydrogen Peroxide,</td> <td>VHP</td> <td>Decontamination</td> <td>Glass</td> </tr> <tr> <td>Modified Vaporous Hydrogen Peroxide</td> <td>mVHP</td> <td><i>B. anthracis</i></td> <td>Silicone</td> </tr> <tr> <td><i>G. stearothermophilus</i></td> <td>CARC</td> <td>Metal</td> <td></td> </tr> </table>						Vaporized Hydrogen Peroxide,	VHP	Decontamination	Glass	Modified Vaporous Hydrogen Peroxide	mVHP	<i>B. anthracis</i>	Silicone	<i>G. stearothermophilus</i>	CARC	Metal	
Vaporized Hydrogen Peroxide,	VHP	Decontamination	Glass														
Modified Vaporous Hydrogen Peroxide	mVHP	<i>B. anthracis</i>	Silicone														
<i>G. stearothermophilus</i>	CARC	Metal															
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON												
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson												
U	U	U	UL	60	19b. TELEPHONE NUMBER (include area code) (410) 436-2914												

Blank

SUMMARY

The STERIS Vaporous Hydrogen Peroxide (VHP®) technology has been used for more than a decade to sterilize pharmaceutical processing equipment and clean rooms. In Oct 2001, the VHP technology was adapted to decontaminate two anthrax-contaminated buildings in the Washington, D.C. area. In 2002, Steris' subsidiary Strategic Technology Enterprises (STE) and the U.S. Army Edgewood Chemical Biological Center (ECBC) began the process to co-develop a modified VHP (mVHP) capable of biological and chemical decontamination. Over the past few years, the mVHP fumigant has been significantly improved for the decontamination of materials contaminated with chemical agents VX, GD, and HD. During this time, the mVHP system was also improved to enable better distribution and higher concentrations. The mVHP technology is widely scalable and adaptable to accommodate a wide range of applications (e.g., buildings, aircraft, and sensitive equipment). Many programs were executed during this time to demonstrate application and determine agent efficacy. Several demonstrations were successfully completed showing large-venue applications and efficacy against agent surrogates. The biological chambers and a biosafety level three (BSL-3) laboratory tests were to determine the decontamination efficacy against biological agent and surrogate on operationally relevant materials. The chemical chambers work was to determine the decontamination efficacy against chemical agents HD, VX, TGD, and GD on operationally relevant materials. This biological chambers and BSL-3 laboratory work is the subject of this report. The work was completed under Contract No. W9115R-04-C-0024, "Mobilization of Three VHP-CB Systems and Evaluation of Impact of Materials on VHP-CB Concentration, Half-Life and Adsorption."

Blank

PREFACE

The work described in this report was authorized under Contract No. W9115R-04-C-0024. The work was started in October 2005 and completed in March 2006.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. Manufacturer names and model numbers are provided for completeness. This report may not be cited for purposes of advertisement.

This report has been approved for public release. Registered users should request additional copies from the Defense Technical Information Center; unregistered users should direct such requests to the National Technical Information Service.

Acknowledgments

A program cannot be successfully completed without the contributions of a good team of people. The authors thank the following individuals for their hard work and assistance with the execution of this technical program:

- Andrew Janick for operating and maintaining the mVHP equipment.
- Dr. David Pawlowski and Elizabeth Tall for processing the biological samples.
- Debbie Cantemiry, Ozzie Goodall, and Damon Smith for their prompt assistance with the Standard Operating Procedure preparation, pre-op, and approval process.
- Ken Younger and his staff at the ECBC Experimental Fabrication Shop for preparing hundreds of tests coupons.
- Larry Oswald of the ECBC Experimental Fabrication Shop, Andrew Janick and Mel Long, Steris, for building a perfect replica of the Steris mVHP Sensitive Equipment Decontamination (SED) Prototype on the 463L pallet for use in this testing.
- Beth DeRego for assisting with the document proofreading.

Blank

CONTENTS

SUMMARY	iii
1. INTRODUCTION	1
1.1 Summary of Conclusions	2
1.2 mVHP® Decontamination Process	3
2. METHODS AND PROCEDURES	6
2.1 Engineering Directorate Chamber Facilities	6
2.2 Decontamination Chamber	6
2.3 Test Materials	7
2.4 Biological Spore Innoculated Test Coupons	7
2.5 Biological Indicators	10
2.6 mVHP Decontamination System Operation	10
2.7 Decontamination Efficacy Targets	11
2.8 Types of Testing	13
3. TEST RESULTS AND DISCUSSION	14
3.1 <i>G. stearothermophilus</i> as Suitable <i>B. anthracis</i> Surrogate for mVHP	14
3.2 Biological Surrogate and Agent Material Recovery Tests	15
3.3 mVHP System Operation	17
3.4 Biological Surrogate Scoping Test (Test B1)	18
3.4.1 Test Summary	18
3.4.2 Enumerated and Qualitative Samples List	18
3.4.3 CT Results	19
3.4.4 BI and Coupon Qualitative Results	19
3.4.5 Enumerated Coupon Results	19
3.4.6 Discussion	19
3.5 Biological Surrogate Efficacy Tests (Tests B2, B3, B6)	20
3.5.1 Test Summary	20
3.5.2 Enumerated and Qualitative Samples List	20
3.5.3 CT Results	20
3.5.4 BI and Coupon Qualitative Results	20
3.5.5 Enumerated Coupon Results	22
3.5.6 Discussion	22
3.6 Biological Surrogate Efficacy Tests at Low Fumigant Concentration (Test B5)	23
3.6.1 Test Summary	23
3.6.2 Enumerated and Qualitative Samples List	23
3.6.3 CT Results	23
3.6.4 BI and Coupon Qualitative Results	24
3.6.5 Enumerated Coupon Results	24

3.6.6	Discussion	24
3.7	Biological Surrogate Baseline Test (Test B4)	24
3.7.1	Test Summary	25
3.7.2	Enumerated and Qualitative Samples List.....	25
3.7.3	CT Results.....	25
3.7.4	BI and Coupon Qualitative Results.....	26
3.7.5	Enumerated Coupon Results.....	26
3.7.6	Discussion	27
3.8	Biological Agent Efficacy Tests	27
3.8.1	Test Summary	27
3.8.2	Coupon Results	27
3.8.3	Discussion.....	28
3.9	Comparison of Coupon Tests to JPID and JSSED ORD Requirements	28
3.10	Spore Loading Visual Comparison.....	29
3.10.1	Test Method	29
3.10.2	Visual Results and Discussion.....	30
3.11	Validation of SED Prototype to Lexan Replica.....	32
3.11.1	Low-Concentration Comparison.....	32
3.11.2	Target-Concentration Comparison.....	33
3.11.3	Summary	34
3.12	Comparison of Two Polycarbonates.....	37
4.	CHALLENGES AND LESSONS LEARNED.....	37
	LITERATURE CITED	39
	GLOSSARY	41
	APPENDIXES	
	A: COUPON STOCK MATERIAL AND PREPARATION	A-1
	B: mVHP CONTROL CHARTS	B-1

FIGURES

1.2.1:	mVHP® Decontamination Chemistry	4
1.2.2:	mVHP® Decontamination Cycle.....	5
2.2:	Lexan Replicate of Steris SED Prototype	8
2.3:	Chemical and Biological Test Coupons.....	9
2.4:	Example of Biological Coupon for Testing	10
2.6:	Biological Test Operation	12
3.2.1:	Spore Recovery Results for <i>G. stearothermophilus</i>	16
3.2.2:	Reproducible Recoveries from Coupon Materials.....	17
3.3:	System Operation	18
3.5.5:	mVHP Efficacy Test Results for <i>G. stearothermophilus</i>	22
3.6.5:	Low-Fumigant Concentration Exposure using <i>G. stearothermophilus</i>	25
3.7.5:	Non-Fumigant Concentration Exposure using <i>G. stearothermophilus</i>	27
3.8.2:	<i>B. anthracis</i> (AMES) Results at Target Fumigant Concentration	28
3.10.1:	Biological Coupon Spore Load Visual Comparison Test.....	30
3.10.2.1:	Spore Loading Visual Comparison	30
3.10.2.2:	10 ⁷ Loading Before and after Wipe Process	31
3.10.2.3:	Enumerated Effects of Wiping on Spore Recovery	32
3.11.1:	Low-Concentration Comparison.....	35
3.11.2:	Target-Concentration Comparison.....	36
3.12:	Comparison of Polycarbonates	37

TABLES

2.7: Operational Requirements Document (ORD) Key Performance Parameters (KPP).....	13
3.4.3: Biological Test CTs -Run B1	19
3.4.4: Qualitative Test Results - Run B1.....	19
3.5.3: Biological Test CTs	21
3.5.4: Qualitative Test Results	21
3.6.3: Biological Test CTs - Run B5.....	23
3.6.4: Qualitative Test Results - Run B5.....	24
3.7.3: Biological Test CTs - Run B4.....	26
3.7.4: Qualitative Test Results - Run B4.....	26
3.11.1: Low-Concentration Half-Life	33
3.11.2: Target-Concentration Half-Life	34

BIOLOGICAL-WARFARE AGENT DECONTAMINATION EFFICACY TESTING: LARGE-SCALE CHAMBER mVHP® DECONTAMINATION SYSTEM EVALUATION FOR BIOLOGICAL CONTAMINATION

1. INTRODUCTION

The STERIS Vaporous Hydrogen Peroxide (VHP®) technology has been used for more than a decade to sterilize pharmaceutical processing equipment and clean rooms.^{1,2} In Oct. 2001, the VHP technology was adapted to decontaminate two anthrax-contaminated buildings in the Washington, D.C. area. In 2002, STERIS Corporation, Inc. subsidiary, Strategic Technology Enterprises (STE), and the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground (APG), MD, began the process to co-develop a modified VHP (mVHP) capable of both biological and chemical decontamination. Over the past few years, the mVHP fumigant has been significantly improved for the decontamination of materials contaminated with chemical agents VX, GD and HD.³ The mVHP technology was developed and patented through a Cooperative Research and Development Agreement (CRADA) between ECBC and STE. During this time, the mVHP system was also improved to enable better distribution and higher concentrations. The mVHP technology is scalable and adaptable to accommodate wide range of applications such as buildings, aircraft and sensitive equipment. Many programs were executed during this time to demonstrate application and determine agent efficacy.⁴ The modular mVHP™ system was successfully demonstrated in a former office building decontamination test at APG, Maryland, and C-141B aircraft decontamination tests at Davis-Monthan AFB in Arizona.⁵⁻⁷ The biological chambers and BSL-3 laboratory work was performed to determine the decontamination efficacy against biological agent and surrogate on operationally relevant materials. The chemical chambers work was performed to determine the decontamination efficacy against chemical agents HD, VX, TGD and GD on operationally relevant materials.⁸ The VHP/mVHP decontamination tests and demonstrations are part of a congressionally funded joint venture between ECBC and STE.

In 2004, a VHP decontamination chamber study using a modified SAMS box showed biological simulant could be decontaminated on sensitive equipment within 4 hr. This finding was the first significant step toward the application of the mVHP technology to the Joint Service Sensitive Equipment Decontamination (JSSSED) program. In June 2005, a sensitive equipment decontamination (SED) prototype was evaluated for operational utility at the Decontamination Limited Objective Experiment (LOE) at Tyndall AFB. The LOE formal report indicated that mVHP has potential applicability for thorough decon of sensitive equipment primarily in rear echelon applications as currently configured on the 463L pallet. Following the LOE, the SED prototype was brought to full decontamination capability. The operational SED prototype was sent to ECBC for sensitive equipment surrogates and biological surrogate decontamination efficacy evaluations.⁹ The prototype used mVHP for chemical- and biological-agent decontamination applications, improved fumigant distribution and delivery methods. The improved methods enabled higher concentrations in field applications. The approach for the chamber chemical agent and biological surrogate testing was to construct a replica of the SED prototype decontamination chamber for use under engineering controls. The use of the replica enabled a complete evaluation of the Steris

mVHP technology: mVHP fumigant, distribution and operating conditions. The replica provided an additional advantage as a tie-point from lab (agent) to field (surrogate) data.

The primary objective of this test was to determine the mVHP system's ability to decontaminate biological-warfare agent contamination on operationally relevant materials. The decontamination efficacy was compared to the Key Performance Parameters (KPPs) stated in the Operational Requirements Document (ORD) for Joint Platform Interior Decontamination (JPID).¹⁰ The decontamination efficacy was compared also to the KPPs stated in the ORD for Joint Service Sensitive Equipment Decontamination (JSSED).¹¹ The tests were performed between October 2005 and March 2006 in the Engineering Directorate large-scale chambers at ECBC. The results for the biological agent and surrogate studies are presented in this report.

1.1 Summary of Conclusions.

This test was conducted to determine the mVHP system ability to decontaminate biological-warfare agent contamination on operationally relevant materials. The summary of conclusions is provided in the bulleted list.

- *B. anthracis* Ames decontamination tests met the ORD equivalent 6-log reduction in viable spores within 5 min of mVHP treatment at 500-ppm hydrogen peroxide and 30-ppm ammonia on operationally relevant materials. (Section 3.10)
- The baseline (no fumigant) and low concentration (250-ppm hydrogen peroxide and 15-ppm ammonia) results did not meet the ORD equivalent 6-log reduction, which was expected. (Section 3.10)
- The 500-ppm hydrogen peroxide and 30-ppm ammonia efficacy tests showed that a 6-log reduction in viable *G. stearothermophilus* spores could be achieved within 30-min for most materials, and within 60-min for all materials. (Section 3.10)
- The time required to achieve a 6-log reduction for *B. anthracis* is far shorter than for *G. stearothermophilus* showing the more conservative nature of *G. stearothermophilus* as an indicator of rendering *B. anthracis* spores nonviable. The results have consistently shown that at 500-ppm hydrogen peroxide and 30-ppm ammonia *G. stearothermophilus* decontamination takes 15 times longer than *B. anthracis* decontamination to achieve the same reduction in viable spores. (Section 3.9)
- A statistical analysis of the chamber test Lexan replica data and the SED prototype data demonstrated that the Lexan replica is statistically equivalent to the SED system prototype. (Section 3.11)
- Thirty minutes of mVHP exposure at 500-ppm hydrogen peroxide and 30-ppm ammonia was sufficient to achieve a 6-log reduction in viable *G. stearothermophilus* spores for most materials. The Chemical Agent Resistant Coating (CARC) coated metal coupons required a slightly longer time closer to 60-min to achieve the same 6-log reduction in viable *G. stearothermophilus* spores. (Section 3.5)
- The low-fumigant concentration (250-ppm hydrogen peroxide, 15-ppm ammonia) results showed that decon chamber temperature, humidity and airflow and sample transport did not result in the loss of spores during the efficacy test. By reducing the fumigant concentration, a larger number of viable spores were recovered. The low-

fumigant concentration test provided a secondary confirmation that the reduction of viable spores observed during the efficacy test was due to the mVHP fumigant concentration. (Section 3.6)

- The baseline results showed that decon chamber temperature, humidity and airflow and sample transport did not result in the loss of spores. The baseline test provided a secondary confirmation that the reduction of viable spores observed during the efficacy test was due to the mVHP fumigant and not to airflow or handling. (Section 3.7)
- A simple pre-wipe step such as a damp cotton swab can reduce the initial challenge by 2-log (Section 3.10).

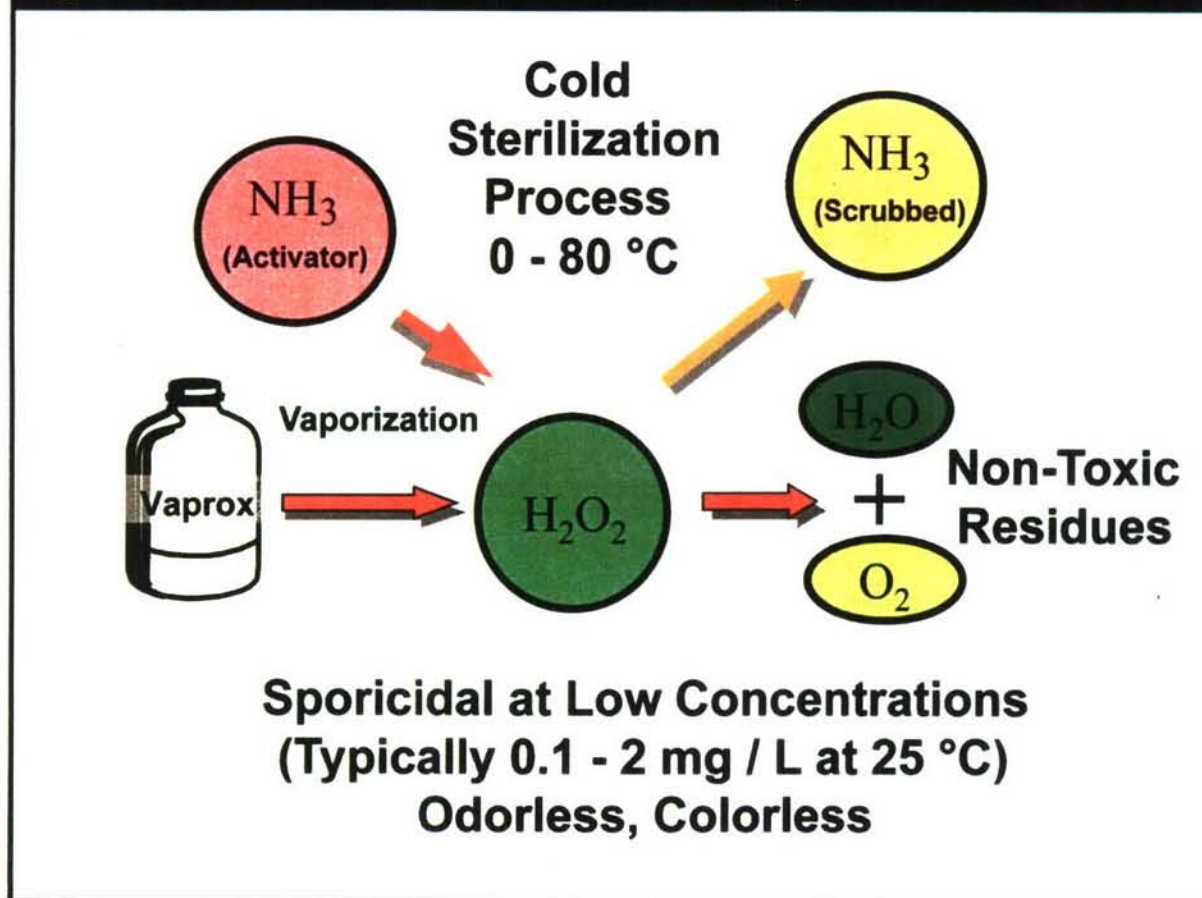
1.2 mVHP® Decontamination Process.

The mVHP is a broad spectrum decontaminant composed of VHP and a small amount of ammonia gas used within a specified set of conditions. The mVHP decontamination process evaluated is the combination of the patented mVHP decontaminant and decontamination operating conditions.^{12,13}

The mVHP decontamination process has been shown effective at atmospheric pressure and at ambient temperatures. The process is completely vapor phase hydrogen peroxide and ammonia. Hydrogen peroxide vapor readily forms hydroxyl free radicals that have been found to react with various micromolecules. VHP rapidly decomposes into two environmentally benign products: oxygen and water vapor (Figure 1.2.1). Metal oxide catalysts are used for large-scale, one-through processes requiring more rapid decomposition on the exhaust stream. The current processes uses up to 30 ppm of ammonia, which is below the Permissible Exposure Limit (PEL) of 50 ppm. Unreacted ammonia is scrubbed out of the exhaust air through an appropriate filter. The large systems monitor the exhaust for ammonia and hydrogen peroxide to ensure no fumigant post the filter bed.

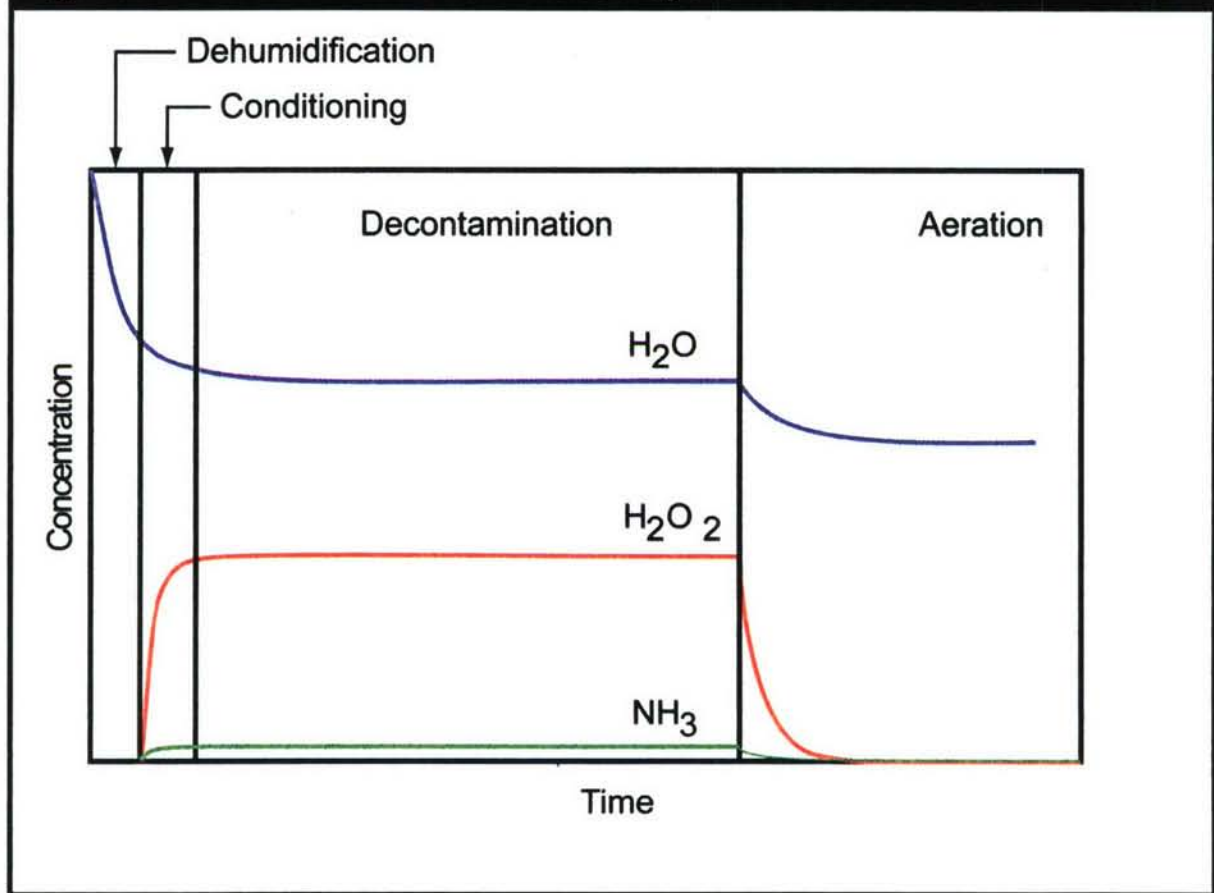
Since mVHP is a vapor technique, the primary requirement for decontamination is an enclosure. The technology is versatile - adaptable to enclosures ranging from defined boxes (e.g., SED), to vehicle and building interiors, to tents.^{4,14}

Figure 1.2.1: mVHP[®] Decontamination Chemistry



Decontamination of an interior/enclosed space using the modular mVHP system is a four-phase process involving preparation of the interior air (dehumidification), achieving a steady state decontaminant level (conditioning), performing the decontamination, and then aerating the space for safe entry (Figure 1.2.2).

Figure 1.2.2: mVHP® Decontamination Cycle



Dehumidification: Hydrogen peroxide vapor can co-condense with water vapor producing an undesired condensate high in hydrogen peroxide. If ambient conditions are likely to permit condensation – high humidity and/or cold temperatures – this can be prevented by circulating dry, heated air through the interior prior to injection of the hydrogen peroxide vapor. The target humidity level is determined by the concentration of vapor to be injected and the desired steady state concentration for the decontamination. The lower relative humidity permits a higher concentration of hydrogen peroxide without reaching a saturation point.

Conditioning: During the conditioning phase, injection of ammonia and hydrogen peroxide vapor is initiated. Injection rates are selected to rapidly raise the concentrations to the desired set point without condensation. Internal sensors measure and report the ammonia and hydrogen peroxide concentrations to the control system. When the concentrations reach the set point values, the ammonia and hydrogen peroxide injection rates are lowered to maintain the set-point concentrations. Once all the interior monitors reach or exceed the set point concentration, the system proceeds to the next phase.

Decontamination: Decontamination is timed-phase dependent on the hydrogen peroxide vapor concentration, ammonia vapor concentration and temperature. A decontamination timer counts down from the preset decontamination time. If the concentrations or temperature values fall below the set point, the timer stops. This ensures that during the decontamination phase, the interior space is exposed to at least the minimum decontamination conditions for the desired exposure time.

Aeration: After completion of the decontamination phase, the system stops injection of hydrogen peroxide and ammonia and introduces only dried air into the interior space. The dried air displaces the hydrogen peroxide and ammonia. The hydrogen peroxide and ammonia are removed by the exhaust system. Samples are drawn and tested from the exhaust system upstream of the catalyst destroyer. When the measurements are below the ammonia and hydrogen peroxide PELs, the user terminates the aeration process.

2. METHODS AND PROCEDURES

2.1 Engineering Directorate Chamber Facilities.

The tests were conducted in one of the Engineering Directorate's large-scale chambers at ECBC. The chamber contained the mVHP decontamination chamber, a working enclosure for sample dosing and the vapor-manifold table. The chamber was monitored using miniCAMs for chemical agent and Drager sensors for ammonia and hydrogen peroxide concentration outside the mVHP decontamination chamber. The filter banks and control areas were also monitored for chemical agent during testing.

2.2 Decontamination Chamber.

A replica of the SED prototype on the 463L pallet decontamination chamber was constructed for use in the ECBC Engineering Directorate Chamber Facility (Figure 2.2). The decontamination chamber provides a test enclosure with a similar volume, dimensions, fumigant distribution, inlet and outlet ports characteristic of the Steris modular mVHP process and the SED prototype. The decontamination chamber is 8-ft long, 4-ft wide and 7-ft tall. The enclosure was constructed from Lexan[®] as two 3.5-ft tall half-boxes. The upper box sits over the lower box to create the decontamination chamber. The SED prototype contains shelves for the placement of equipment. The chamber replica has a stainless steel table fitted with a stainless steel mesh top for placement of the coupon containers. The SED prototype decontamination chamber is accessed via doors on the narrow sides of the unit. The use of full-size doors was not practical for the chamber testing since the tests focused on the required decontamination phase time. Opening a large door would result in higher loss of fumigant as samples were removed during the decontamination phase. The replica has two ports of entry: an access door and a small "pizza oven" door. The samples are placed in, and removed from, the decontamination chamber via the pizza oven door.

2.3 Test Materials.

The selected test materials span a variety of structural and functional materials common to aircraft, vehicles, protective- and sensitive-equipment that encompass a variety of material properties, compositions and porosities. The test materials include bare aluminum, CARC-painted aluminum, AF-topcoat-painted aluminum, glass, polycarbonate, Viton[®], Kapton[®] and silicone (Figure 2.3). The biological agent surrogate test coupons are 1.3 cm squares, except glass, which is round. The chemical agent test coupons are 2-in. circular disks with a surface area of 3.14 in.² (20.27 cm²).⁸ The glass chemical agent test coupons were ordered pre-cut from McMaster-Carr. All other chemical and biological test coupons were cut from stock material. Uniformity is assured by obtaining a large enough quantity of material that multiple test samples can be prepared with uniform characteristics (e.g., test coupons will all be cut from the interior rather than the edge of a large piece of material). All coupons are stored in zip-tight bags in containers to prevent/limit contact with foreign substances until the coupons are needed for testing. The biological test coupons were sterilized prior to use. The coupon preparation information including material vendors and descriptions is provided in Appendix A.

2.4 Biological Spore Innoculated Test Coupons.

G. stearotherophilus spore stocks were purchased from Apex Laboratories, Apex, NC (lot 329251 and product number LPT-606). Coupons were sterilized in small Petri dishes with wire mesh screens in groups of 4 per dish. They were autoclaved for 25 min at 121 °C and 15 psi. Once the Petri dishes were cooled, the surface of each coupon was inoculated with a 10-μL volume 1×10^6 , 1×10^7 , or 1×10^8 spores in water. The spore-inoculated coupons were left in a biosafety level two hood until they appeared visibly dry prior to testing. Once dry, the dishes with coupons were transferred to Tupperware containers and transported to the chamber for experiments. After the exposure, samples are transported back to the laboratory in Tupperware containers. One of each coupon type for each timepoint was aseptically transferred to 5 mL Tryptic Soy Broth (TSB) and incubated at 55 °C. Coupons were observed daily for 7 days. If positive growth was detected (turbid broth) the remaining 3 coupons were processed. Coupons were aseptically placed in 5 mL buffered peptone water with 0.01% Tween 80 and sonicated for 10 min. Following sonication, 10 μl of Antifoam 289 was added and samples were vortexed at maximum speed for 2 min. Samples were then serially diluted in buffered peptone water and pour plated (1 mL per plate) using Tryptic Soy Agar (TSA). Plates were gently swirled in each direction and allowed to solidify in a biosafety cabinet. Once solidified, plates were transferred to a 55 °C incubator overnight. Resultant colonies were enumerated the following day. A representation of the biological coupons in Petri dishes and Tupperware containers is shown in Figure 2.4.

Figure 2.2: Lexan Replicate of Steris SED Prototype

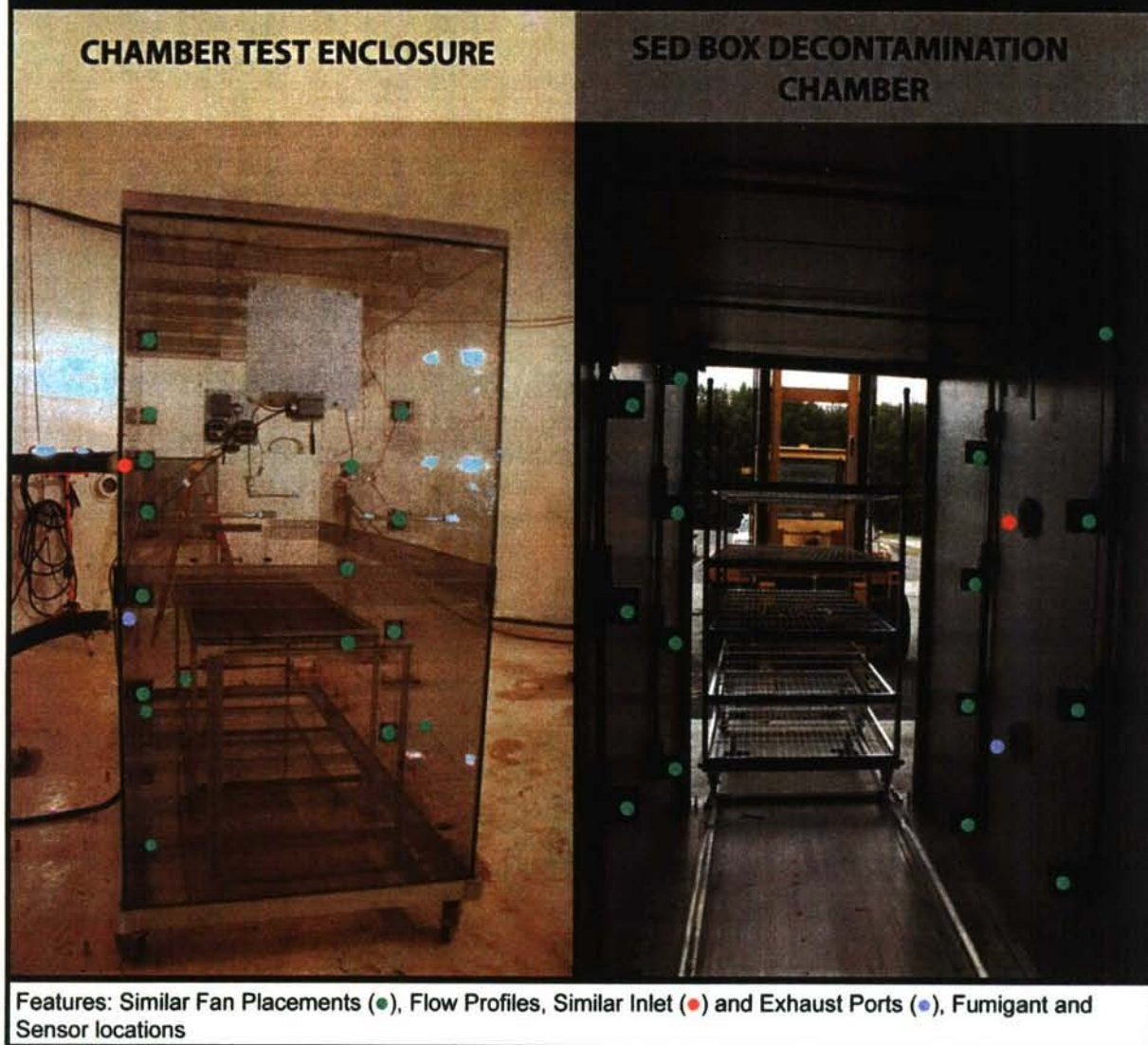


Figure 2.3: Chemical and Biological Test Coupons



Figure 2.4: Example of Biological Coupon for Testing



2.5 Biological Indicators.

Commercial *G. stearothermophilus* spore biological indicators (BIs) functioned as a confirmatory test for sporicidal effectiveness. The commercial BIs, inoculated to a level of approximately 10^6 colony forming units (CFUs), were purchased from two vendors, Apex (ATCC 12980, Lot H2165 Exp. 31 March 06) and STERIS (ATCC 7953, Lot 1885B Exp. April 7, 06). *G. stearothermophilus* was specifically selected for testing since it is a spore forming bacterium that has been identified as an appropriate conservative surrogate for *B. anthracis* with the VHP technology. After exposure, BIs were transported back to laboratory with coupons in Tupperware containers. In the laboratory, BIs were aseptically transferred to 5 mL TSB broth and incubated for 7 days at 55 °C. Samples were checked daily and considered non-viable after 7 days if no turbidity (growth) was observed.

2.6 mVHP Decontamination System Operation.

A Munter's dehumidifier was used to maintain the relative humidity within the decontamination chamber. Vaprox brand certified 35% hydrogen peroxide was used for the generation of the hydrogen peroxide vapor. Ammonia gas lecture bottles were used to supply the ammonia. The conditioning phase was a two part process. The first portion of conditioning was the rapid injection of hydrogen peroxide and ammonia into the chamber to achieve the target concentrations. Once the target concentration was achieved, the second part of the conditioning process was maintaining the target concentrations for at least 20 min. The beginning of the decontamination phase for this test program is defined as the time the coupons were placed in the enclosure. The target concentrations were maintained throughout the decontamination phase.

The coupons were placed into the decontamination chamber via the small pizza oven door (Figure 2.6). The Tupperware dishes were removed from the transport container, placed on a work table and the Tupperware lids removed. The Tupperware dish was placed into the

decontamination chamber. Using a fresh set of gloves, the test operator uncovered the Petri dish, placing the lid next to coupon dish inside the Tupperware dish. The test operator slid the Tupperware into the chamber using an extension pole. At each exposure time, the appropriate Tupperware dishes were pulled forward, the Petri dish and Tupperware dish lids were secured and then the Tupperware dish was brought out via the pizza oven door. After the final coupons were removed from the decontamination chamber at the last exposure time, the introduction of fumigant was stopped and the system proceeded into the aeration phase.

2.7 Decontamination Efficacy Targets.

The determination of decontamination efficacy is measured by quantifying the amount of agent (or surrogate) remaining after a decontamination process and comparing to the agent (or surrogate) starting amount. The decontamination efficacy value can typically be expressed in terms of the percent agent (or surrogate) reduction resulting from the decontamination process. The mVHP technology study has evaluated the potential application of the technology to interior decontamination. In May 2005, the Joint Platform Interior Decontamination (JPID) Operational Requirements Document (ORD) was issued specifying threshold and objective key performance parameters (KPP) for thorough decontamination efficacy for chemical vapor- and contact-hazards, and biological agent residual levels.¹¹ In spring 2005, the development of the SED prototype added the evaluation of the technology for the potential application to sensitive equipment. The potential application to sensitive equipment falls under the ORD for the Joint Service Sensitive Equipment Decontamination (JSSED) program Joint Service Interior Decontamination (JSID) document. The JSSED ORD document also specifies threshold and objective KPPs for thorough decontamination efficacy for chemical vapor- and contact-hazards and biological agent residual levels.¹² The JPID and JSSED ORD KPP values are listed in Table 2.7. The evaluation results were compared to both ORD KPPs as applicable.

Figure 2.6: Biological Test Operation

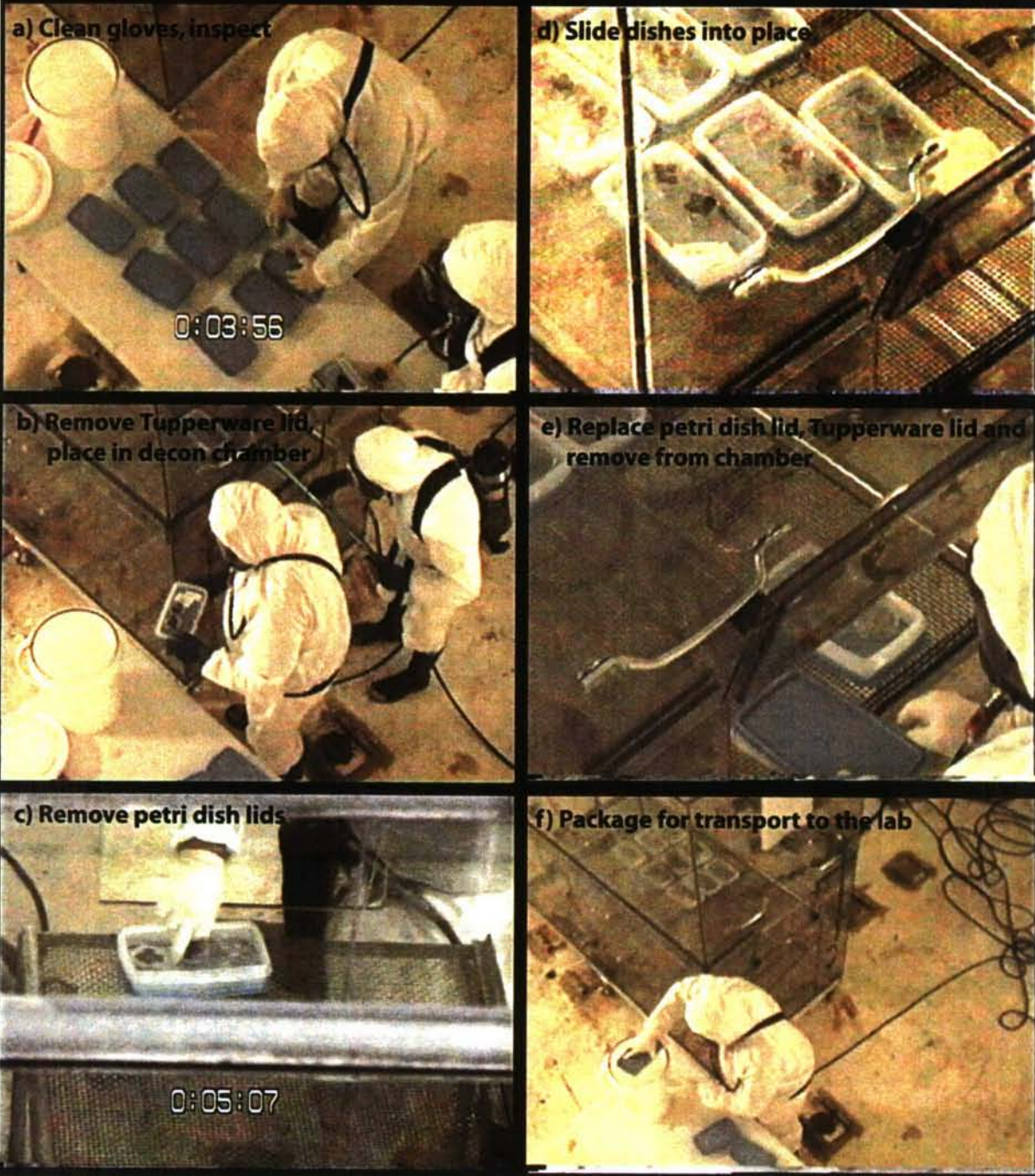


Table 2.7: Operational Requirements Document (ORD) Key Performance Parameters (KPP)

VAPOR HAZARD	Starting Challenge	Nerve - G	Nerve - V	Blister - H
JPID Threshold Vapor Level	1 g/m ²	< 0.00087 mg / m ³	< 0.000036 mg / m ³	< 0.0058 mg / m ³
JPID Objective Vapor Level	1 g/m ²	< 0.0002 mg / m ³	< 0.000024 mg / m ³	< 0.003 mg / m ³
JSSSED Threshold Vapor Level	10 g/m ²	< 0.1 mg / m ³	< 0.04 mg / m ³	< 0.1 mg / m ³
JSSSED Objective Vapor Level	10 g/m ²	< 0.0001 mg / m ³	< 0.00001 mg / m ³	< 0.003 mg / m ³
CONTACT HAZARD	Starting Challenge	Nerve - G	Nerve - V	Blister - H
JPID Threshold Exposure Level	1 g/m ²	< 1.7 mg / m ²	< 0.04 mg / m ²	< 3.0 mg / m ²
JPID Objective Exposure Level	1 g/m ²	0.0 mg / m ²	0.00 mg / m ²	0.0 mg / m ²
JSSSED Objective Exposure Level	10 g/m ²	< 16.7 mg / m ²	< 0.78 mg / m ²	< 100 mg / m ²
BIOLOGICAL	Starting Challenge	Bacterial Endospores	Vegetative Bacteria	Viruses
JPID Threshold Reduction	1x10 ⁸ CFU/m ²	< 100 CFU / m ²	< 10 CFU / m ²	< 10 PFU / m ²
JSSSED Objective Reduction	Not specified	< 100 CFU / m ²	< 10 CFU / m ²	< 10 PFU / m ²

2.8 Types of Testing.

Engineering Test: The engineering test was conducted to verify the mVHP system can achieve and maintain the target 500-ppm hydrogen peroxide- and 30-ppm ammonia-concentrations for 10 hr. Tupperware containers were loaded onto the stainless steel table to mimic the test configuration. Each container had at least one chemical indicator strip to verify that fumigant contacted the inside area of each Tupperware dish. The results of the engineering test are documented in the chemical agent result report.⁸

Biological Surrogate and Agent Material Recovery Tests: The biological surrogate and agent material recovery tests were conducted prior to the decontamination tests to determine the spore recovery efficiency from the various substrates. Procedures are modified as needed to enable spore recovery efficiency. No modifications were necessary for the materials used in this test.

Biological Surrogate Scoping Test: The coupon contamination starting challenge concentration is 1 x 10⁶ spores per coupon. The mVHP decontaminant was used. The scoping test fumigant concentrations are 500-ppm hydrogen peroxide and 30-ppm ammonia. Samples were collected at selected time-points and processed. The time-point showing complete kill was designated as the end-point. The scoping tests determine the sample collection times (exposure times) for the efficacy tests.

Biological Surrogate Efficacy Test: The efficacy tests use contaminated coupons and biological indicators. The coupon contamination starting challenge concentration is 1×10^6 spores per coupon. The mVHP decontaminant was used. Two efficacy test fumigant concentrations were used: 500-ppm hydrogen peroxide/30-ppm ammonia and 250-ppm hydrogen peroxide/15-ppm ammonia. Based on the scoping tests, samples were collected at regular intervals starting at time of preparation (time-point 0) through the scoping test end-point.

Biological Surrogate Baseline Test: The efficacy tests use contaminated coupons and biological indicators. The coupon contamination starting challenge concentration is 1×10^6 spores per coupon. The mVHP decontaminant was not used. Air was passed over the coupons for the duration of the test. The baseline provides information regarding the impact of air flow on spore removal.

Biological Agent Efficacy Test: The efficacy tests use contaminated coupons. The coupon contamination starting challenge concentration is 1×10^6 spores per coupon. The mVHP decontaminant was used. The biological agent *B. anthracis* Ames was used. The efficacy test fumigant concentrations are 500-ppm hydrogen peroxide and 30-ppm ammonia. These tests were conducted in the ECBC BSL-3 laboratory.

Biological Starting Contamination Comparison Test: The comparison tests address the series of contamination starting challenge concentrations: 1×10^6 (ECBC Methods), 1×10^7 (JPID) and 1×10^8 (JSSED) spores per coupon. The comparison tests address the challenges associated with decontamination of significantly high loadings (1×10^7 and 1×10^8). The comparisons are made visually and quantitatively before and after the pre-wipe. Select comparisons were made with the biological surrogate, *G. stearothermophilus*, and biological agent, *B. anthracis* Ames.

3. TEST RESULTS AND DISCUSSION

3.1 *G. stearothermophilus* as Suitable *B. anthracis* Surrogate for mVHP.

The selection of an appropriate simulant for biological agent warfare decontamination can be strongly influenced by the active component of the decontaminant to be used. A suitable simulant for the mVHP evaluation should react similar to *Bacillus anthracis*. In addition, the simulant should be more conservative than the actual agent. The simulant should be rendered non-viable in either the same time or longer than the actual agent. The same- or delayed-time effect would enable that the determined simulant decontamination cycle times are more than sufficient for the actual agent decontamination.

Laboratory work conducted early in the mVHP test programs addressed the selection of biological simulant in comparison to *B. anthracis* strains. *Geobacillus stearothermophilus* was found to be the best simulant for *B. anthracis* with VHP/mVHP.

3.2 Biological Surrogate and Agent Material Recovery Tests.

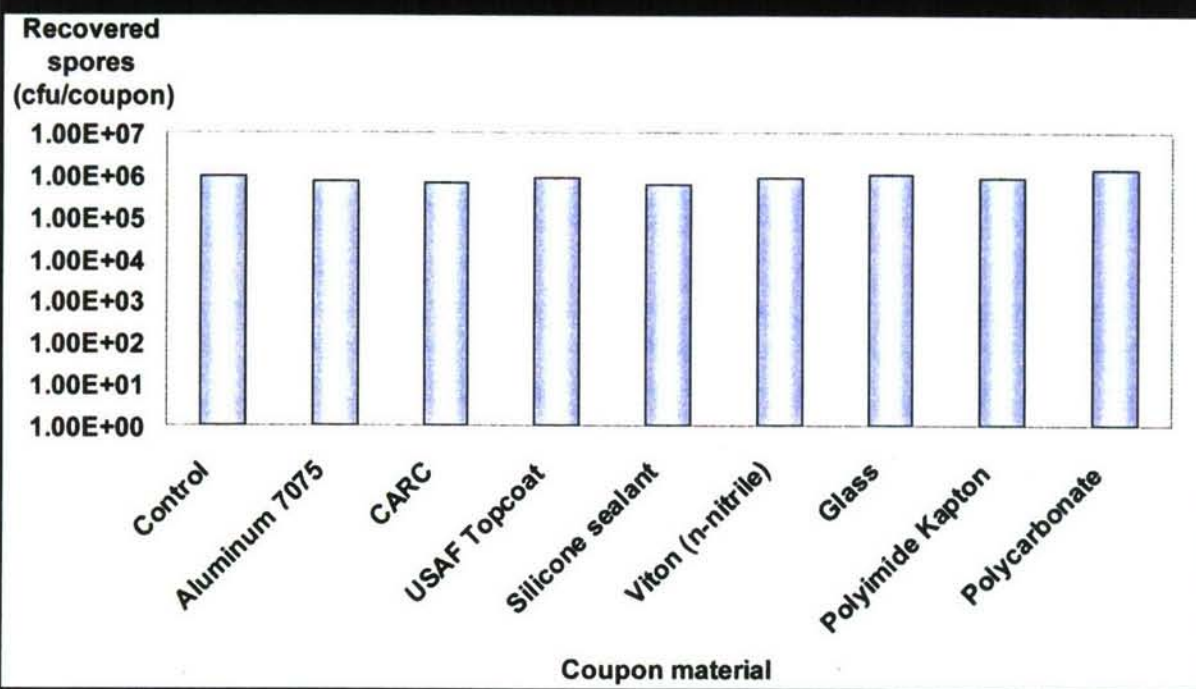
The recovery tests were originally developed based upon a test plan first put in place in Feb. 2003. Organisms were applied to coupon materials in a 5% Fetal Bovine Serum/Buffered Peptone Water solution to simulate bioburden. At the time, a total of 1×10^7 spores in a 10 μ l solution were loaded onto coupons because recovery efforts only yielded 1×10^6 . The decision was made to load 1×10^7 spores so that 1×10^6 spores would be recovered each time. After further experimentation in the laboratory, an updated recovery method was developed. The FBS was dropped from the inoculum due to discrepancies in the data. The spores were purchased from Apex Laboratories to prevent any inconsistencies in lot to lot variation and 0.01% Tween 80 was added to increase recovery rate. As a result of adding 0.01% Tween 80 to recovery media, the inoculum amount was decreased because almost 100% recovery rate was achieved. The inoculum amount currently used is 1×10^6 spores in 10 μ l buffered peptone water per coupon. The averaged results for the material recovery tests are provided in Figure 3.2.1.

For each test a control set of samples was prepared and kept in the laboratory. The control samples were processed using the same method as the mVHP exposed samples. The results for each control set are shown as "time 0" for each run. The control values show that the recovery method enables a 1×10^6 spore recovery from the coupon surfaces.

The reproducibility of the spore recoveries can be demonstrated by comparing the control samples from the chambers and SED test programs. In addition, the baseline chambers and SED program results were used in this analysis. The results are shown in Figure 3.2.2.

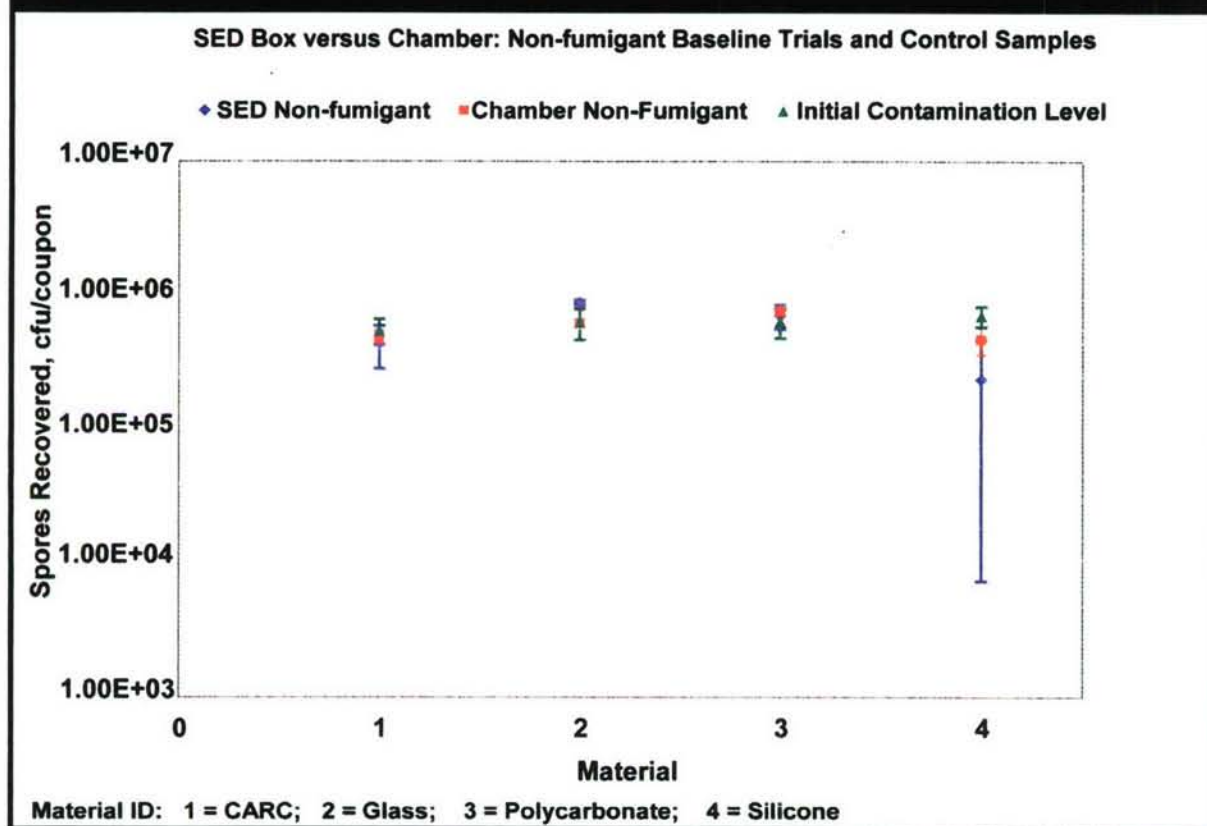
A statistical analysis of the data from the two test chambers was conducted, using the Q-test for statistical outliers, and Student's t-test to compare groups. Within the individual test groups of coupon materials, there were no statistical outliers, despite data scatter that generated standard deviations between 2% (polycarbonate, chamber) and 97% (silicone, SED Box) of the mean value of the concentration.

Figure 3.2.1: Spore Recovery Results for *G. stearothermophilus*



Student's t-test was calculated using the data from similar coupons in the two chambers to determine if there were statistically significant differences between the performances of the two chambers. For all four coupon materials, the Student's t-test values calculated using the two-tailed, heteroscedastic values with four degrees of freedom are unable to reject the hypothesis that the two data sets are statistically identical at the $p=0.1$ significance level. Therefore, the spore recovery of the SED prototype is not statistically different from the spore recovery of the chamber test system.

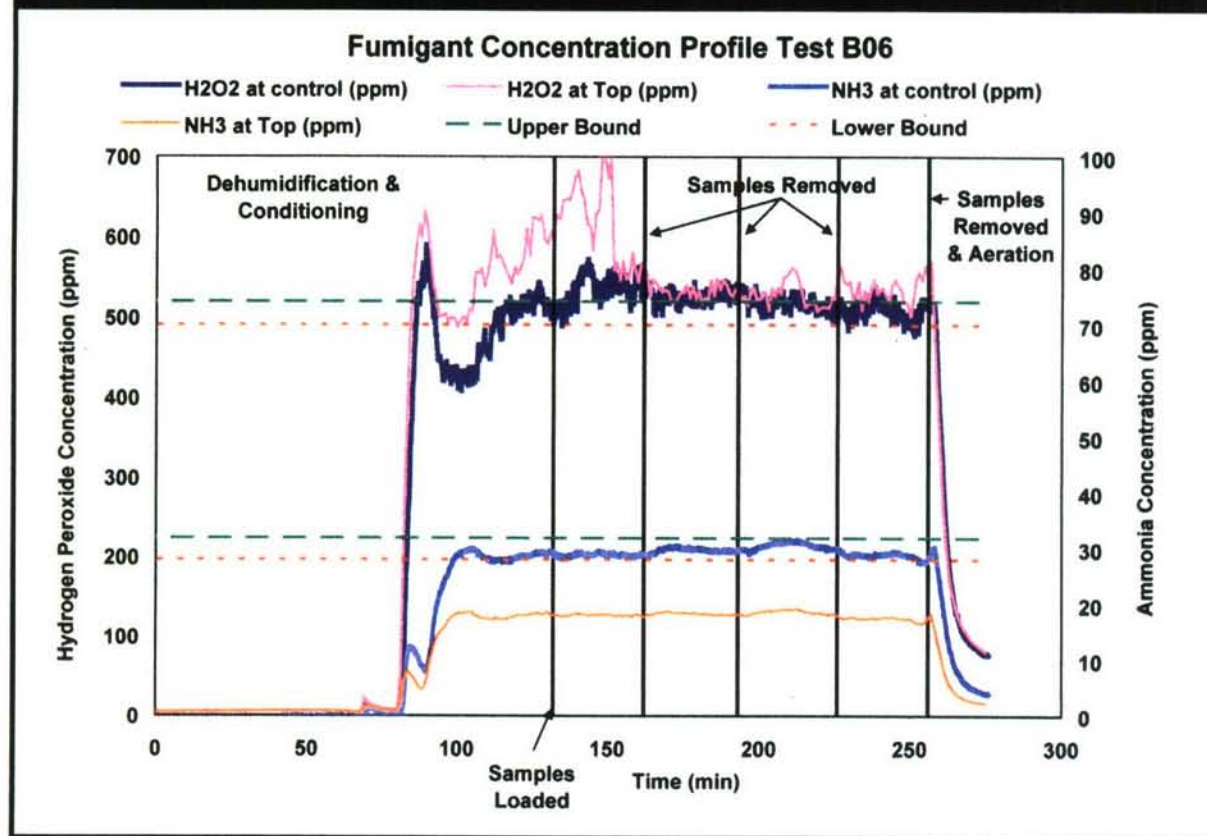
Figure 3.2.2: Reproducible Recoveries from Coupon Materials



3.3 mVHP System Operation.

The mVHP decontamination process is a four-phase process. The sample loading, sample removal and process phases are shown in Figure 3.3. The system was operated such that dehumidification and conditioning were initiated without samples in the decon chamber. The samples were loaded after the fumigant concentration was maintained at or above target for approximately 30-min. The samples were loaded into the box and then removed at selected intervals. The control charts for each run showing fumigant concentration, temperature, humidity, sample loading and removal are provided in Appendix B.

Figure 3.3: System Operation



3.4 Biological Surrogate Scoping Test (Test B1).

3.4.1 Test Summary.

The scoping test used contaminated coupons and biological indicators. The coupon starting challenge was 1×10^6 spores per coupon, which is equivalent to 5.9×10^9 cfu/m². The mVHP decontaminant was used. The efficacy test target fumigant concentration was 500-ppm hydrogen peroxide and 30-ppm ammonia. The scoping tests used contaminated coupons and 2 biological indicators for each incubation time. Samples were collected at 0- (control), 2-, 4- and 6-hr and processed. The hydrogen peroxide and ammonia fumigant concentrations, temperature and relative humidity control charts are provided in Appendix B.

3.4.2 Enumerated and Qualitative Samples List.

All four of the "time 0" replicates for each material type were plated for enumeration. The 2-, 4- and 6-hour sample analysis was only qualitative broth samples. If the samples had shown growth (i.e. positive broth response), then the remaining three replicates would have been plated. The BIs were placed in broth for the qualitative determination.

3.4.3 CT Results.

The time for sample loading and removal, fumigant concentration setpoint and calculated CT are provided in Table 3.4.3.

Table 3.4.3: Biological Test CTs - Run B1					
Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	10:09 a.m.	500	0	30	0
2-hour pull at	12:09 p.m.	500	1000	30	62
4-hour pull at	2:10 p.m.	500	2041	30	127
6-hour pull at	4:12 p.m.	500	3107	30	189

3.4.4 BI and Coupon Qualitative Results.

The scoping test qualitative coupon and BI results showed no growth (Table 3.4.4).

Table 3.4.4: Qualitative Test Results - Run B1			
Material	Exposure Time		
	2-hour	4-hour	6-hour
Glass	-	-	-
Polycarbonate	-	-	-
CARC	-	-	-
USAF TopCoat	-	-	-
Aluminum	-	-	-
Viton	-	-	-
Silicone	-	-	-
Polyimide (Kapton)	-	-	-
BI - Apex	-	-	-
BI - Steris	-	-	-

3.4.5 Enumerated Coupon Results.

The qualitative coupon results in Section 3.4.4 show that there were no viable spores at any of the sample points. No samples were plated. The efficacy tests were conducted using a maximum decontamination time of 2 hr.

3.4.6 Discussion.

The scoping test was to determine the approximate time required to achieve non-viable spores on all material types. The scoping test showed that all spores were rendered non-viable on all

material types within 2-hr. The subsequent efficacy tests would use shorter times to determine the time required for thorough decontamination efficacy.

3.5 Biological Surrogate Efficacy Tests (Tests B2, B3, B6).

3.5.1 Test Summary.

The efficacy tests used contaminated coupons and biological indicators. The coupon challenge was 1×10^6 spores per coupon, which is equivalent to 5.9×10^9 cfu/m². The mVHP decontaminant was used. The efficacy test target fumigant concentration was 500-ppm hydrogen peroxide and 30-ppm ammonia. Based on the scoping tests, samples were collected at time of preparation (time-point 0), 30, 60, 90 and 120-min. Two replicate tests were performed to demonstrate repeatability of the test and results. The cumulative CTs for each time-point for each test are provided in Tables 3.5.3. The hydrogen peroxide and ammonia fumigant concentrations, temperature and relative humidity controls charts are provided in Appendix B.

3.5.2 Enumerated and Qualitative Samples List.

All four of the “time 0” replicates for each material type were plated for enumeration. The 30-, 60-, 90- and 120-min sample treatment was first the qualitative broth test. Samples showing growth (i.e. positive broth response) were plated for enumeration. The BIs were placed in broth for the qualitative determination.

3.5.3 CT Results.

The time for sample loading and removal, fumigant concentration setpoint and calculated CT for the three efficacy runs are provided in Table 3.5.3.

3.5.4 BI and Coupon Qualitative Results.

The efficacy test qualitative coupon results showed a range of responses based on material type. The CARC samples showed growth at the 90-min time-point. Viton showed growth at the 60-min time-point in the replicate test run B6. AF-topcoat, aluminum and Kapton showed some growth at 30 min. At the 120-min time-point, growth was not observed on any of the material types. The qualitative results for the initiate test (B2 and B3) and the replicate test (B6) are provided in Table 3.5.4. The Apex and Steris BIs showed no growth.

Table 3.5.3: Biological Test CTs

Run B2

Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	9:42 a.m.	500	0	30	0
30 minute pull at	10:12 a.m.	500	259	30	15
60 minute pull at	10:43 a.m.	500	517	30	32
90 minute pull at	11:14 a.m.	500	770	30	47
120 minute pull at	11:45 a.m.	500	1029	30	63

Run B3

Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	9:15 a.m.	500	0	30	0
30 minute pull at	9:44 a.m.	500	251	30	14
60 minute pull at	10:15 a.m.	500	506	30	29
90 minute pull at	10:47 a.m.	500	777	30	45
120 minute pull at	11:18 a.m.	500	1038	30	60

Run B6

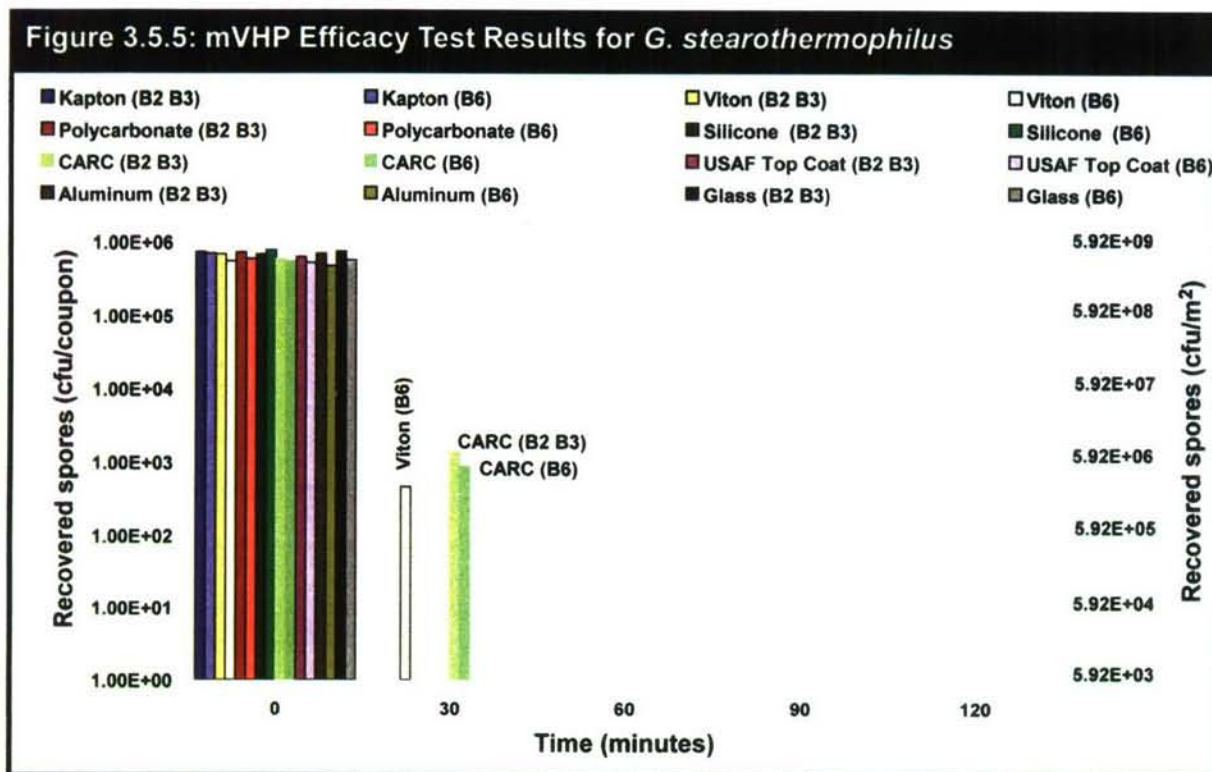
Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	10:08 a.m.	500	0	30	0
30 minute pull at	10:38 a.m.	500	269	30	14
60 minute pull at	11:09 a.m.	500	541	30	30
90 minute pull at	11:42 a.m.	500	825	30	47
120 minute pull at	12:12 p.m.	500	1079	30	61

Table 3.5.4: Qualitative Test Results

Material	Exposure Time, Minutes							
	30-minutes		60-minutes		90-minutes		120-minutes	
	Run B2/B3	Run B6	Run B2/B3	Run B6	Run B2/B3	Run B6	Run B2/B3	Run B6
Glass	+	-	-	-	-	-	-	-
Polycarbonate	-	-	-	-	-	-	-	-
CARC	+	+	+	+	+	+	-	-
USAF TopCoat	+	+	-	-	-	-	-	-
Aluminum	-	+	-	-	-	-	-	-
Viton	+	+	-	+	-	-	-	-
Silicone	-	-	-	-	-	-	-	-
Polyimide (Kapton)	+	-	-	-	-	-	-	-
BI - Apex	-	-	-	-	-	-	-	-
BI - Steris	-	-	-	-	-	-	-	-

3.5.5 Enumerated Coupon Results.

The first efficacy tests, run B2 and B3, showed *G. stearotheophilus* growth on CARC at 30-min (Figure 3.5.5). The replicate test, run B6, showed *G. stearotheophilus* growth on CARC and Viton at 30-min.



3.5.6 Discussion.

Thirty minutes of mVHP exposure at 500-ppm hydrogen peroxide and 30-ppm ammonia was sufficient to achieve a 6-log reduction in viable *G. stearotheophilus* spores for most materials. The CARC coated metal coupons required a slightly longer time closer to 60-min to achieve the same 6-log reduction in viable *G. stearotheophilus* spores.

Enumerated coupon tests confirmed that <10 colony forming units (cuff) were viable on the replicate coupon materials treated for 30-min. The positive growth observed on CARC at the 60- and 90-min time-points in the qualitative coupon analysis coupled with the lack of growth on the comparable enumerated coupons, indicates that <10 survivors were responsible for the cloudiness observed in the qualitative test.

3.6 Biological Surrogate Efficacy Tests at Low Fumigant Concentration (Test B5).

Based on the large amount of kill at the target 500-ppm hydrogen peroxide and 30-ppm ammonia efficacy conditions, a second efficacy test was conducted at a lower fumigant concentration to demonstrate that at lower concentration, higher growth counts are obtained for the same exposure time-points. This low concentration test also provided confidence in the efficacy test results at the target concentration. The low concentration efficacy tests used contaminated coupons and biological indicators.

3.6.1 Test Summary.

The efficacy tests used contaminated coupons and biological indicators. The coupon challenge was 1×10^6 spores per coupon, which is equivalent to 5.9×10^9 cfu/m². The mVHP decontaminant was used. The efficacy test target fumigant concentration was 250-ppm hydrogen peroxide and 15-ppm ammonia. Based on the scoping tests, samples were collected at time of preparation (time-point 0), 30-, 60-, and 120-min. The hydrogen peroxide and ammonia fumigant concentrations, temperature and relative humidity controls charts are provided in Appendix B.

3.6.2 Enumerated and Qualitative Samples List.

All four of the “time 0” replicates for each material type were plated for enumeration. The 30-, 60- and 120-min CARC, silicone, glass and polycarbonate samples were initially evaluated using qualitative broth test. Samples showing growth (i.e., positive broth response) were plated. The Viton, Kapton, aluminum and AF-topcoat four replicate samples were placed in broth for the qualitative determination. The BIs were placed in broth for the qualitative determination.

3.6.3 CT Results.

The time for sample loading and removal, fumigant concentration setpoint and calculated CT are provided in Table 3.6.3.

Table 3.6.3: Biological Test CTs - Run B5					
Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	12:44 p.m.	250	0	15	0
30 minute pull at	1:14 p.m.	250	127	15	8
60 minute pull at	1:45 p.m.	250	263	15	15
120 minute pull at	2:46 p.m.	250	517	15	31

3.6.4 BI and Coupon Qualitative Results.

The low-fumigant concentration qualitative coupon results all showed growth; whereas, the Steris BIs showed no growth and the Apex BIs showed no growth beyond 60 min (Table 3.6.4).

Table 3.6.4: Qualitative Test Results - Run B5			
Material	Exposure Time		
	30-minutes	60-minutes	120-minutes
Glass	+	+	+
Polycarbonate	+	+	+
CARC	+	+	+
USAF TopCoat	+	+	+
Aluminum	+	+	+
Viton	+	+	+
Silicone	+	+	+
Polyimide (Kapton)	+	+	+
BI - Apex	+	-	-
BI - Steris	-	-	-

3.6.5 Enumerated Coupon Results.

The low-fumigant concentration test showed *G. stearothermophilus* survivors on CARC, silicone and glass at each sampling time-point. The polycarbonate results had no survivors by the 120-min sample. The samples were dosed at 1×10^6 cfu/coupon, which was 5.92×10^9 cfu/m². The results for the low-fumigant concentration tests in cfu/coupon and cfu/m² are shown in Figure 3.6.5.

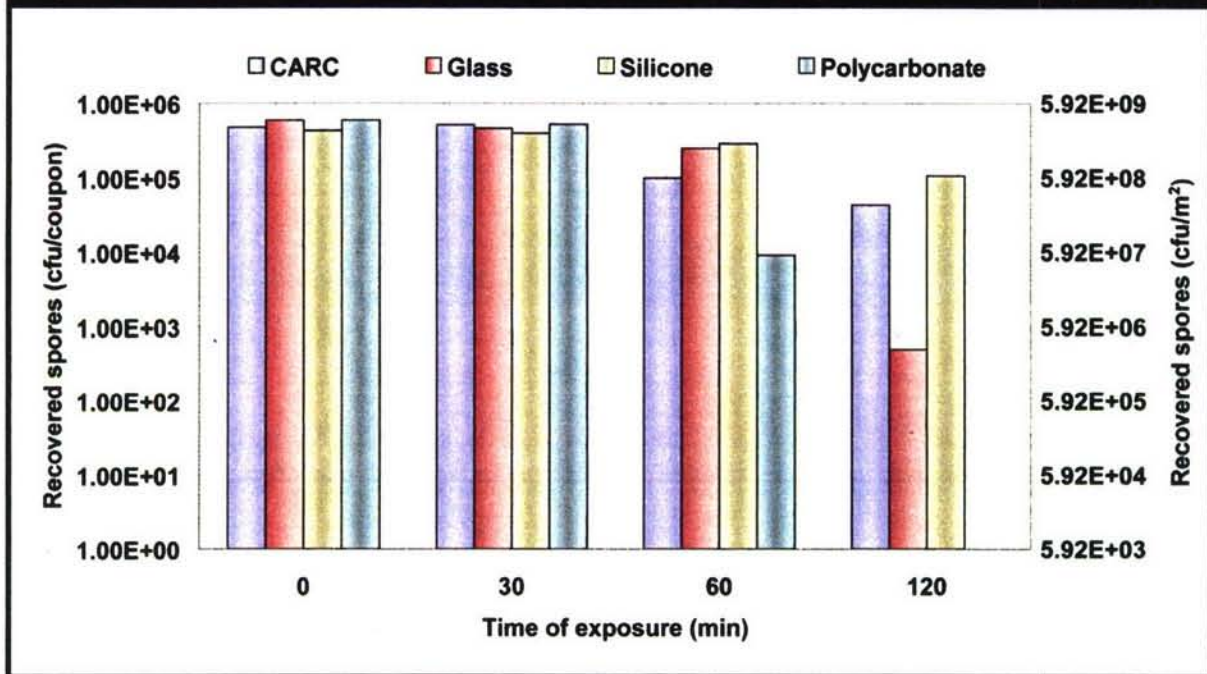
3.6.6 Discussion.

The low-fumigant concentration results showed a significantly larger number of viable spores. The low-concentration test results demonstrated that the mVHP process conditions temperature, humidity and air flow and sample transport did not result in the loss of spores during the efficacy test due to physical removal.

3.7 Biological Surrogate Baseline Test (Test B4).

Based on the large amount of kill at the target 500-ppm hydrogen peroxide and 30-ppm ammonia efficacy conditions, a third efficacy test was conducted without fumigant to demonstrate that without fumigant, higher growth counts are obtained for the same exposure time-points. This “no fumigant” test also provided confidence in the efficacy test results at the target concentration by demonstrating that spores were not lost during sample transport or by decon chamber airflow. The baseline tests utilized both contaminated coupons and biological indicators.

Figure 3.6.5: Low-Fumigant Concentration Exposure using *G. stearotherophilus*



3.7.1 Test Summary.

The baseline tests used contaminated coupons and biological indicators. The coupon challenge was 1×10^6 spores per coupon, which is equivalent to 5.9×10^9 cfu/m². The mVHP decontaminant was not used. Based on the scoping tests, samples were collected at time of preparation (time-point 0) and 120-min. The hydrogen peroxide and ammonia fumigant concentrations, temperature and relative humidity controls charts are provided in Appendix B.

3.7.2 Enumerated and Qualitative Samples List.

All four of the “time 0” replicates for each material type were plated for enumeration. The 120-min CARC, silicone, glass, and polycarbonate samples were plated for enumeration. The Viton, Kapton, aluminum, and AF-topcoat four replicate samples were placed in broth for the qualitative determination. The BIs were placed in broth for the qualitative determination.

3.7.3 CT Results.

Fumigant was not used during the baseline test resulting in zero CTs for the runs. The time for sample loading and sample removal are provided in Table 3.7.3.

Table 3.7.3: Biological Test CTs - Run B4

Activity	Time	Hydrogen Peroxide Conc. (ppm)	Hydrogen Peroxide CT (ppm - hr)	Ammonia Concentration (ppm)	Ammonia CT (ppm - hr)
Samples Loaded	9:37 a.m.	0	0	0	0
30-minute pull at	10:07 a.m.	0	0	0	0
60-minute pull at	10:38 a.m.	0	0	0	0
120-minute pull at	11:39 a.m.	0	0	0	0

3.7.4 BI and Coupon Qualitative Results.

Fumigant was not used during the baseline test. The qualitative BI and coupon results all showed growth (Table 3.7.4).

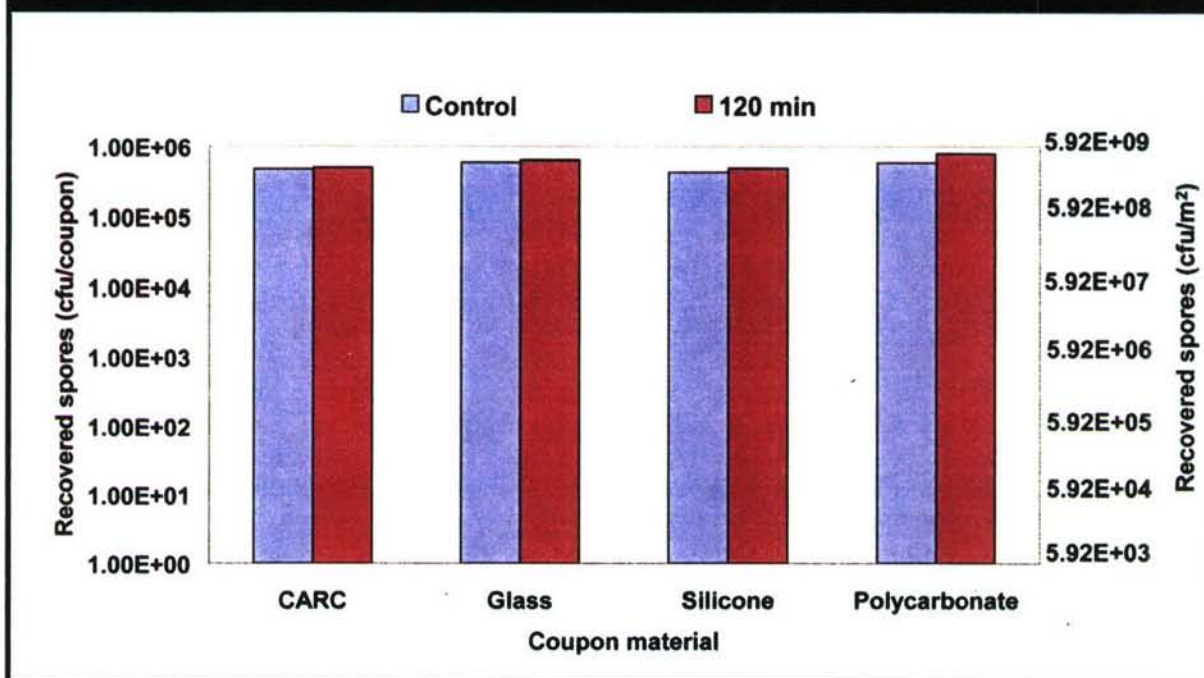
3.7.5 Enumerated Coupon Results.

The baseline test showed *G. stearothermophilus* recoveries for the control and 120-min samples. The samples were dosed at 1×10^6 cfu/coupon, which is 5.92×10^9 cfu/m². The results for the baseline tests in cfu/coupon and cfu/m² are shown in Figure 3.7.5.

Table 3.7.4: Qualitative Test Results - Run B4

Material	Exposure Time		
	30-minutes	60-minutes	120-minutes
Glass	+	+	+
Polycarbonate	+	+	+
CARC	+	+	+
USAF TopCoat	+	+	+
Aluminum	+	+	+
Viton	+	+	+
Silicone	+	+	+
Polyimide (Kapton)	+	+	+
BI - Apex	+	+	+
BI - Steris	+	+	+

Figure 3.7.5: Non-Fumigant Concentration Exposure using *G. stearothermophilus*



3.7.6 Discussion.

The baseline results showed that decon chamber temperature, humidity and air flow and sample transport did not result in the loss of spores. The baseline test provided a secondary confirmation that the reduction of viable spores observed during the efficacy test was due to the mVHP fumigant and not to airflow or handling.

3.8 Biological Agent Efficacy Tests.

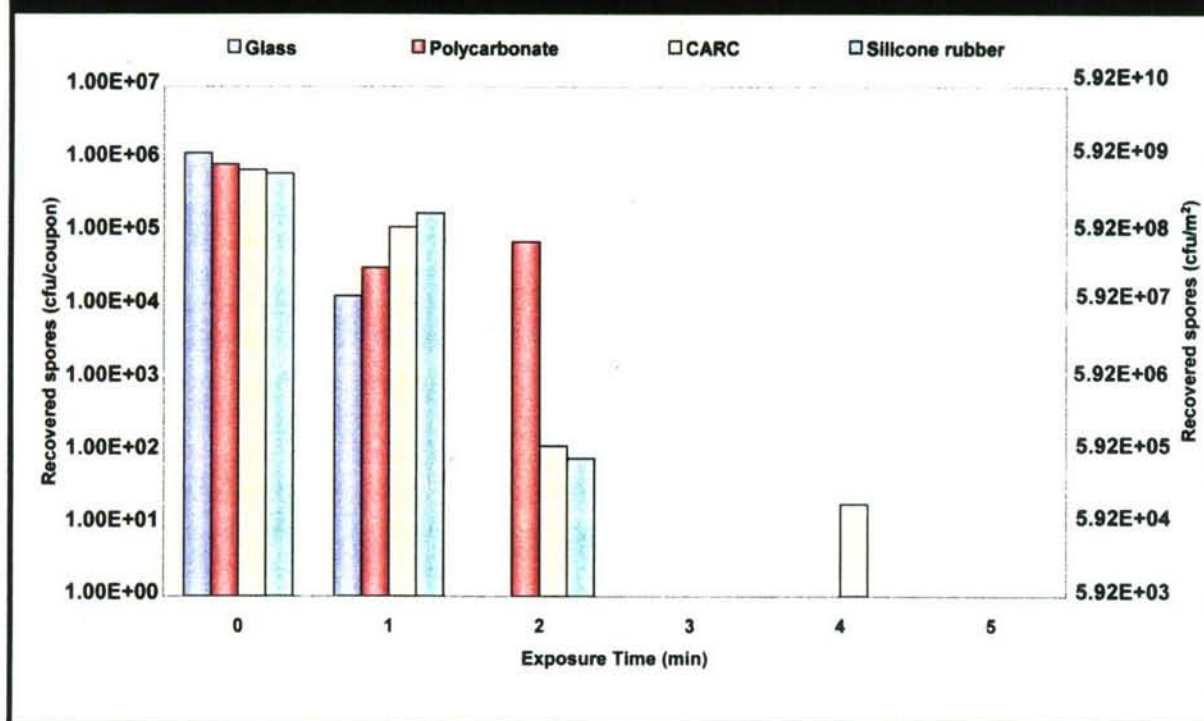
3.8.1 Test Summary.

The biological agent efficacy tests used contaminated coupons at the same challenge of 1×10^6 spores per coupon (which is equivalent to 5.9×10^9 cfu/m²) as the biological surrogate tests. The biological agent efficacy tests are required to demonstrate the effectiveness of fumigant against live agent. The mVHP decontaminant was used. The efficacy test target fumigant concentration was 500-ppm hydrogen peroxide and 30-ppm ammonia.

3.8.2 Coupon Results.

The *B. anthracis* Ames results showed that for three of the four core materials, the 6-log reduction was achieved within 3 min of mVHP exposure. The CARC-coated metal 6-log reduction was achieved by 5 min of mVHP exposure.

Figure 3.8.2: *B. anthracis* (AMES) Results at Target Fumigant Concentration



3.8.3 Discussion

The time required to achieve a 6-log reduction for *B. anthracis* is far shorter than for *G. stearothermophilus* showing the more conservative nature of *G. stearothermophilus* as an indicator of rendering *B. anthracis* spores nonviable. The results have consistently shown that at 500-ppm hydrogen peroxide and 30-ppm ammonia *G. stearothermophilus* decontamination takes 15 times longer than *B. anthracis* decontamination to achieve the same reduction in viable spores.

3.9 Comparison of Coupon Tests to JPID and JSSED ORD Requirements.

The JPID ORD specifies a starting challenge of 1×10^8 cfu/m². Both ORDs specify the remaining contamination to be ≤ 100 cfu/m². The ORDs require a 6-log reduction in viable spores to achieve decontamination.

The tests followed standard procedures for biological coupon testing. These tests use small coupons measuring 1.3 cm by 1.3 cm. When the tests were first started, there was concern that the amount loaded on the coupon was not comparable to the ORD. The standard procedure uses a load of 1×10^6 cfu/coupon. Accounting for coupon area, the initial load is equivalent to 5.9×10^9 cfu/m². The challenge used in the standard procedure is greater than what is required.

The existing test method is based on cfu per coupon. The test was conducted to determine if a 6-log reduction could be achieved with the mVHP technology. The results are presented in

terms of log reduction. The 500-ppm hydrogen peroxide and 30-ppm ammonia efficacy tests showed that a 6-log reduction in viable *G. stearothermophilus* spores could be achieved within 30 min for most materials, and within 60 min for all materials. The *B. anthracis* Ames decon tests met the ORD equivalent 6-log reduction in viable spores within 5 min of mVHP treatment at 500-ppm hydrogen peroxide and 30-ppm ammonia. The baseline (no fumigant) and low concentration (250-ppm hydrogen peroxide and 15-ppm ammonia) results did not meet the ORD equivalent 6-log reduction, which was expected.

In terms of actual number of spores, the 6-log reduction specified by the JPID ORD is equivalent to the removal of 100,000,000 cfu. The existing test method cannot quantify 100 cfu/m² since that is equivalent to 0.017 cfu/coupon. One of the proposed test improvements is to be able to quantify the equivalent of 100 cfu/m². In terms of absolute numbers, the JPID ORD is equivalent to the removal of 100,000,000 cfu. The 500-ppm hydrogen peroxide/30-ppm ammonia results show a reduction in spores that is three-orders of magnitude greater than the ORD required reduction. The reduction in spores on silicone, CARC, glass, and polycarbonate were on the order of 1,300,000,000; 6,000,000,000; 5,000,000,000; and 5,000,000,000; respectively.

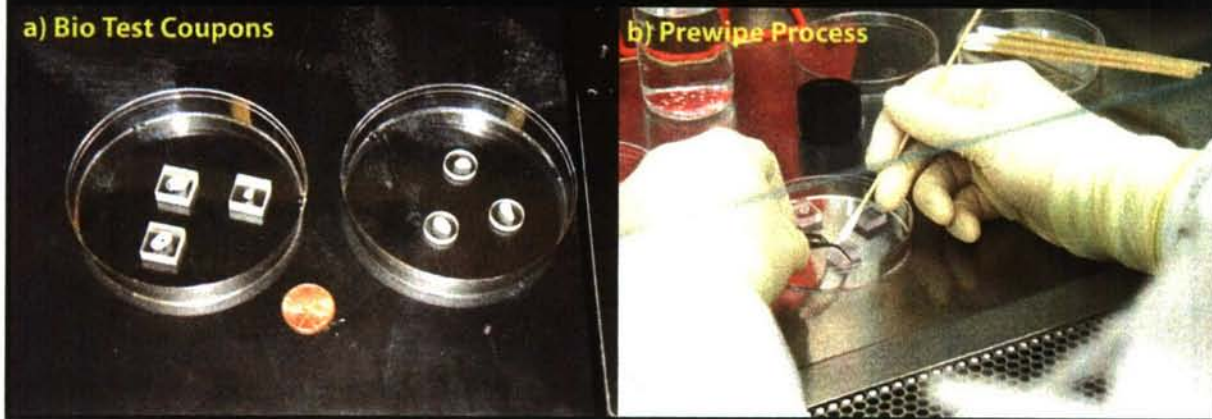
3.10 Spore Loading Visual Comparison.

When the first test matrix was developed, a concern was expressed that the amount loaded onto the coupons was not sufficient to meet the JPID ORD starting challenge of 1×10^8 spores per square meter. A thorough process analysis was conducted, which resulted in the finding that the units in the historical testing were not the same as the ORD. The test method uses 1×10^6 spores per coupon, which is equivalent to 5.9×10^9 spores per square meter. Having resolved that the current protocol uses more spores than required by the ORD, the need for side by side testing at higher loadings was not needed. This section will visually display what these different challenge levels look like.

3.10.1 Test Method.

Polycarbonate and glass samples were used for the analysis. The bio test coupons are slightly smaller than a penny (Figure 3.11.1 left photo). The samples were inoculated with the appropriate concentration of spores and allowed to dry in a bio-safety cabinet before photographing. For the wipe evaluation, the samples were wiped using a damp cotton swab, allowed to dry and then photographed.

Figure 3.10.1: Biological Coupon Spore Load Visual Comparison Test



3.10.2 Visual Results and Discussion.

The ORD starting challenge is 1.0×10^8 cfu/m², which is similar to the 1.0×10^4 cfu/coupon load. At this load, the contamination is not clearly visible to the naked eye without specifically looking for the inoculation on the coupon (Figure 3.10.2). As the load is increased, spores are clumped higher, resulting in clearly visible contamination on the surface.

Figure 3.10.2.1: Spore Loading Visual Comparison



A repeat test was done at the 1.0×10^7 cfu coupon and 1.0×10^8 cfu/coupon starting challenge to show visually and quantitatively the reduction in spores after a pre-wipe. This test was important prior to the unit problem to demonstrate that a 1.0×10^8 cfu/m² load could be reduced to 1.0×10^6 cfu/m² with a prewipe. After the discovery that the 1.0×10^6 cfu/coupon is actually greater than 1.0×10^8 cfu/m², this issue was no longer important. Since the work was completed, it is being provided in the report for completeness. Two sets of samples were prepared at 1.0×10^7 cfu/coupon. One set was handled for the photography the other was kept sterile for enumeration. A single set of samples were prepared at 1.0×10^8 cfu/coupon load for enumeration. A representative glass and polycarbonate coupon at 1.0×10^7 cfu/coupon shown in Figure 3.10.2.2. The pre-wipe was then applied to both sets. The set previously photographed was photographed post the pre-wipe (Figure 3.10.2.2). The other set was enumerated (Figure 3.10.2.3). The results showed that a 2-log reduction in spores was achieved using solely a damp cotton swab.

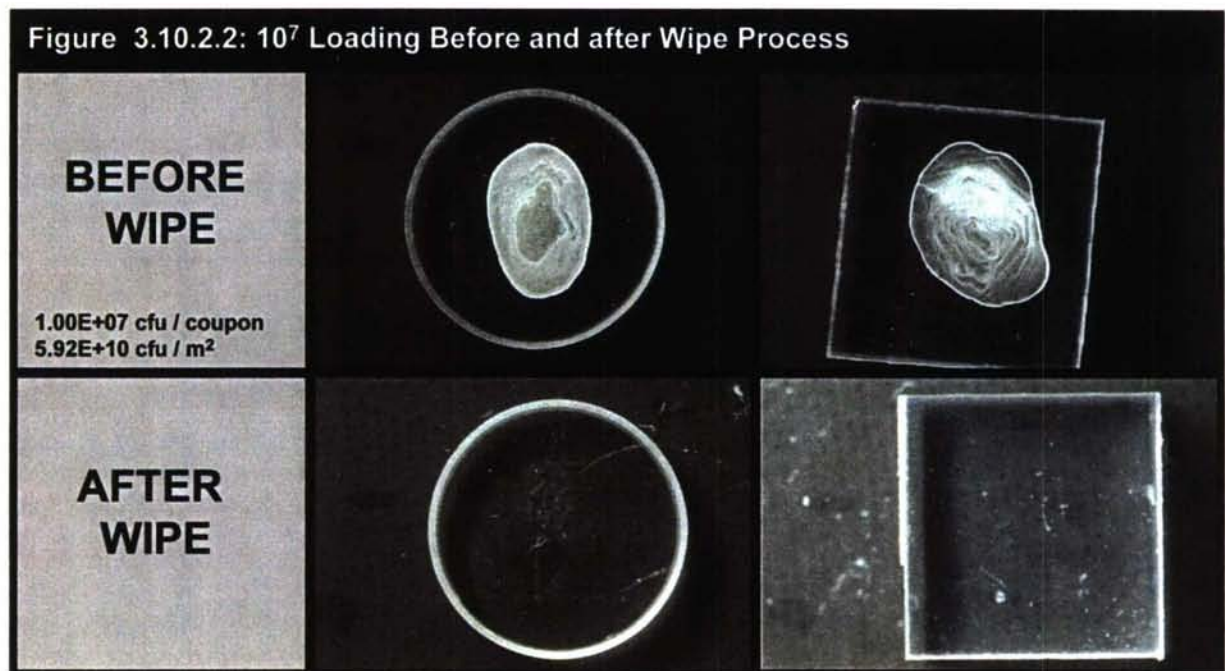
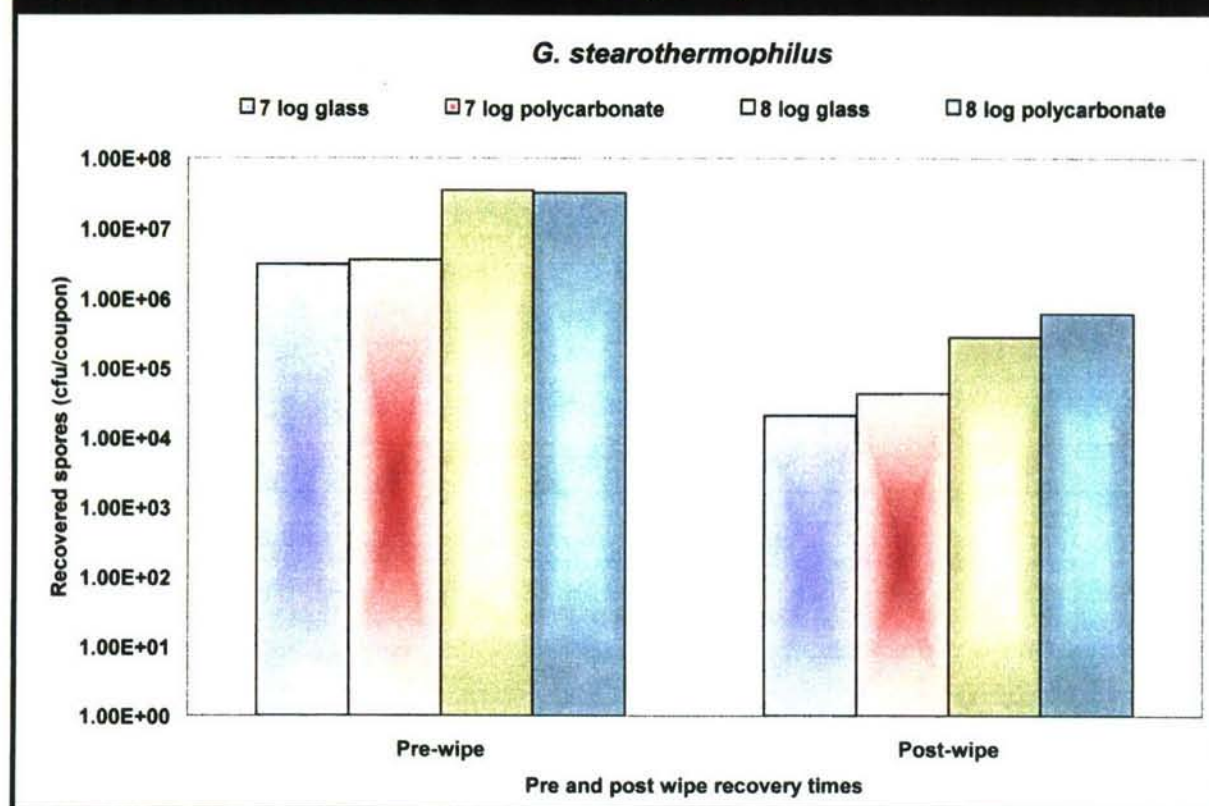


Figure 3.10.2.3: Enumerated Effects of Wiping on Spore Recovery



3.11 Validation of SED Prototype to Lexan Replica.

Chamber and SED prototype fumigant tests were conducted at two different concentrations: 250-ppm hydrogen peroxide (H_2O_2) with 15-ppm ammonia (NH_3) [hereafter abbreviated as 250/15], and 500-ppm H_2O_2 with 30-ppm NH_3 [500/30].

The first comparison of the data for each concentration is between the temporal response and the concentration-time (CT) value, respectively, of the two chambers with respect to their efficacy on the four coupon materials. This evaluation was performed to show that the results obtained between the Chamber and SED prototype are comparable, thus validating the Chamber Lexan replica as representative of the mVHP SED prototype.

3.11.1 Low-Concentration Comparison.

The time-based efficacy results for 250/15 against the biological surrogate *G. stearothermophilus* are provided in Figure 3.11.1a. The differing sample access methods of the two test chambers make it difficult to remove coupons at precisely matching exposure times and CTs. The Chamber system was accessible at user-selectable intervals for withdrawal of sample coupons at pre-designated exposures; whereas, the SED prototype needed to run the full four-phase cycle before samples could be removed for analysis. While

the individual sets of data cannot be compared directly from here, it is evident that the peroxide is destroying the contaminant. The data was then compared in terms of CT, which was calculated by integrating the concentration of peroxide over the decontamination time period (Figure 3.11.1b).

Based on CT, the 80-min SED samples were exposed to almost the identical CT value as the 60-min chamber samples. The CT value for the SED prototype samples was 251 ppm-hr of peroxide, and the chamber CT value was 258 ppm-hr (a difference of 2.6%). Each chamber had one data point that could have been rejected as an outlier, but was retained due to the small sample size. The Student's t-test compared the results for identical coupon materials from the two chambers. The t-test results are unable to reject the hypothesis at the $p = 0.1$ significance level that the data from the two chambers was statistically identical. Within the limits of the sample size, no statistically significant difference in the performance of the SED prototype and the Chambers test box can be detected under these test conditions.

Assuming that the fumigation/decontamination is a 1st order process, exponential trend lines were calculated for the various data sets that compare the different rates of destruction of the biological surrogate for the two test chambers. The exponential fits and corresponding half-life ($t_{1/2}$) values for *G. stearothermophilus* are listed in Table 3.11.1. From the available data, it appears that the half-life of *G. stearothermophilus* on the various test coupons in the SED prototype is comparable to that of the surrogate coupons in the Lexan chamber test box under the test conditions. This further reinforces the premise that the two chambers are functioning in a comparable manner.

Table 3.11.1: Low-Concentration Half-Life

Coupon Material	SED Prototype			Lexan Box		
	Equation	R ²	$t_{1/2}$ (min)	Equation	R ²	$t_{1/2}$ (min)
CARC-painted	$y = 5.7 \times 10^5 e^{-0.004x}$	1.00	173	$y = 6.5 \times 10^5 e^{-0.006x}$	0.9	126
Glass	$y = 6.6 \times 10^5 e^{-0.012x}$	1.00	58	$y = 2.0 \times 10^6 e^{-0.014x}$	0.86	50
Polycarbonate	$y = 6.6 \times 10^5 e^{-0.005x}$	1.00	136	$y = 1.0 \times 10^6 e^{-0.015x}$	0.92	46
Silicone	$y = 7.3 \times 10^5 e^{-0.026x}$	1.00	27	$y = 6.5 \times 10^5 e^{-0.003x}$	0.97	231

3.11.2 Target-Concentration Comparison.

The time-based efficacy results for 500/30 against the biological surrogate *G. stearothermophilus* are provided in Figure 3.11.2a. The low- concentration test had significant contamination remaining on all the coupons after 2 hr (only 1- to 3-logs killed); whereas, the target-concentration test spore concentration was reduced to below the minimum detection limit (MDL) within 90 min (5- to 6-logs killed). Only the CARC-painted coupon has any surviving spores after 50 min of exposure, while in most cases all the spores are destroyed within 30 min. The 500/30 concentration exposure is significantly more effective at destroying *G. stearothermophilus* spores on the four coupon surfaces than the 250/15

setting. Figure 3.11.2b compares the CT response of the systems. Again, CARC-painted aluminum appears to be slightly more difficult to decontaminate than other materials.

In this case, there are no data sets that are directly comparable with regards to the CT value as there were for the 250/15 trials. The Chamber Lexan box appears to be rendering the *G. stearothermophilus* non-viable more rapidly than the SED prototype under the 500/30 fumigation conditions. Table 3.11.2 presents the exponential fit for the different rates of destruction of the biological surrogate for the two test chambers, along with the respective half-lives.

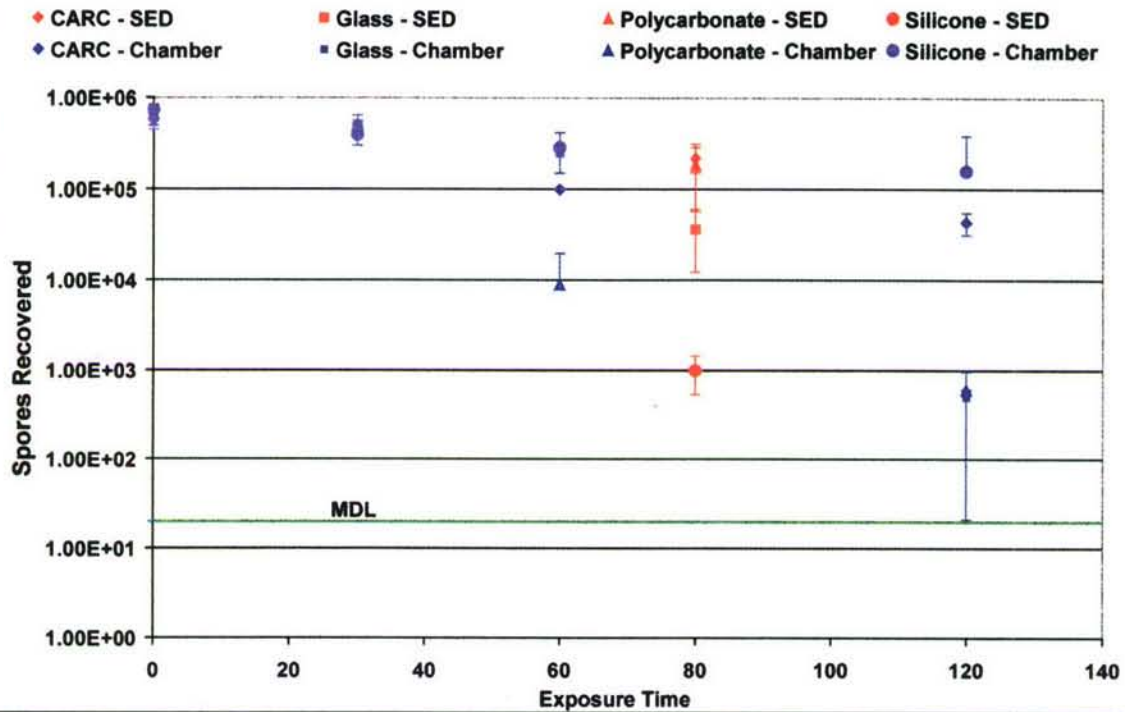
Table 3.11.2: Target-Concentration Half-Life						
Coupon Material	SED Prototype			Lexan Box		
	Equation	R ²	t _{1/2} (min)	Equation	R ²	t _{1/2} (min)
CARC-painted	$y = 6.6 \times 10^5 e^{-0.015x}$	0.96	46	$y = 5.6 \times 10^5 e^{-0.023x}$	1.00	30
Glass	$y = 4.1 \times 10^5 e^{-0.015x}$	0.88	46	$y = 6.6 \times 10^5 e^{-0.039x}$	1.00	18
Polycarbonate	$y = 3.7 \times 10^5 e^{-0.015x}$	0.88	46	$y = 6.6 \times 10^5 e^{-0.039x}$	1.00	18
Silicone	$y = 1.1 \times 10^5 e^{-0.013x}$	0.88	53	$y = 7.3 \times 10^5 e^{-0.039x}$	1.00	18

3.11.3 Summary.

From this data, *G. stearothermophilus* in the SED prototype has about twice the half-life of *G. stearothermophilus* in the Lexan box at the 500/30 conditions. The two units only differed in operational flow rate, the SED unit was limited by the exhaust filter to 20 cfm; whereas, chamber flow was 40 cfm. Within the experimental limits of our tests, and taking into account the different materials of construction and variations, such as flowrate, the SED prototype and the Lexan test chamber appear to provide comparable results.

Figure 3.11.1: Low-Concentration Comparison

a) Plot in Terms of Time



b) Plot in Terms of CT

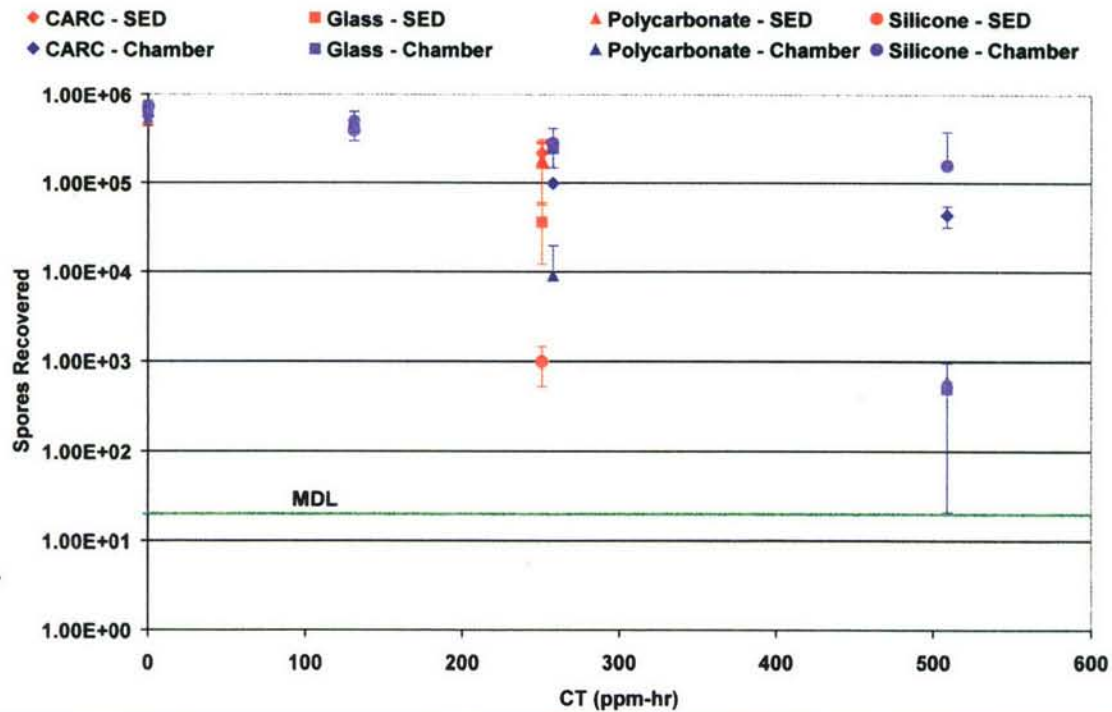
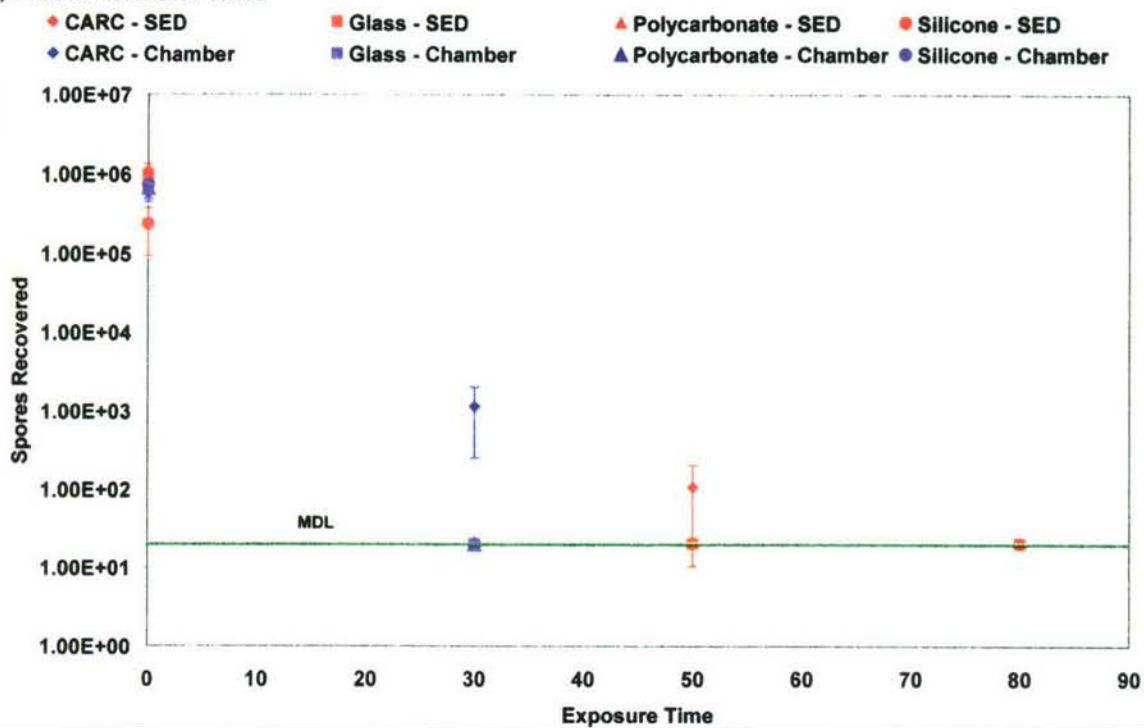
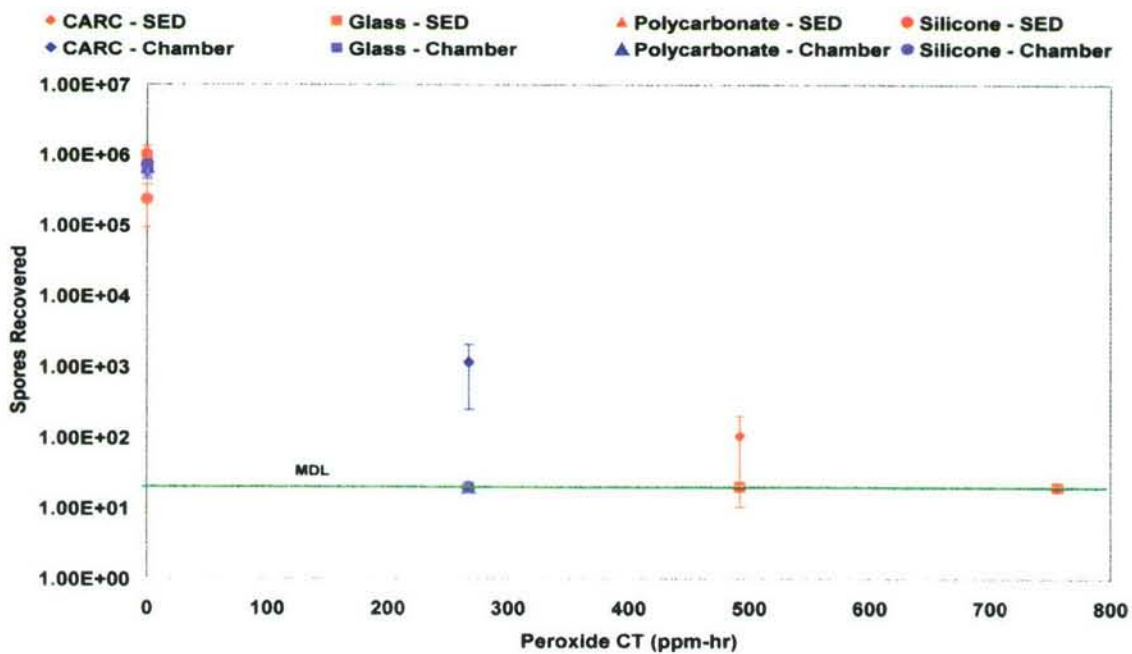


Figure 3.11.2: Target-Concentration Comparison

a) Plot in Terms of Time

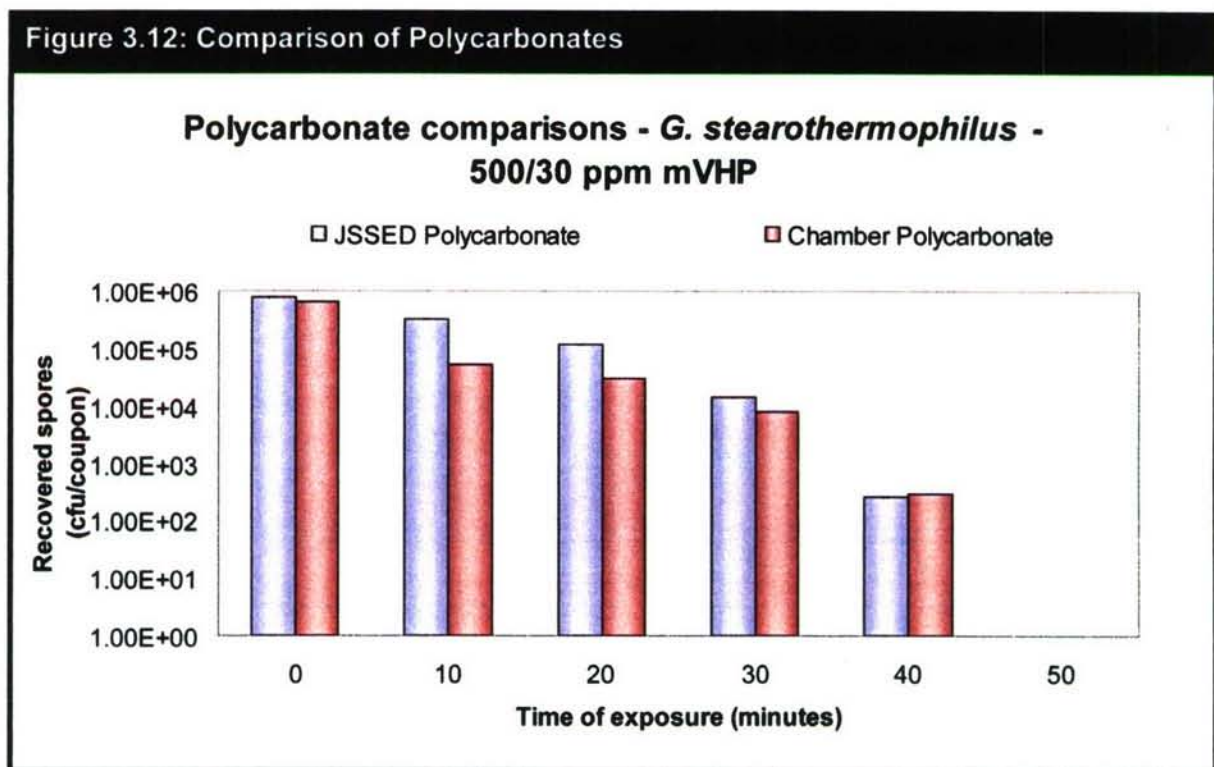


b) Plot in Terms of CT



3.12 Comparison of Two Polycarbonates.

During the test plan development there was interest from the JPEO-JPM Decon working group to use coupon retains from the ECBC Engineering Directorate JSSED program to provide a link to prior program testing. The samples were not available at the time of the chamber tests so the polycarbonate originally acquired for the testing was used. A side-by-side lab test was conducted later to show that the ECBC JSSED polycarbonate performed similar to the chamber polycarbonate (Figure 3.12).



4. CHALLENGES AND LESSONS LEARNED

Always Check the Units.

Several years of testing had been conducted prior to this program. In all of those previous studies on this topic the units of measure were colony forming units (cfu) per coupon. Depending on the program, that coupon size could vary. For this program, the coupon size has been constant. The earlier programs have compared results to the JPID ORD. That ORD specifies results in cfu per square meter (cfu/m²).

During this program a thorough data review discovered this discrepancy between the laboratory methods and the ORD requirements. The error benefited and debited the laboratory testing. The benefit was that the standard procedure applies more spores to the

coupon surface than required by the ORD starting challenge. The debit was that the counting technique could not count reliability <10 cfu. Samples containing no viable spores as identified with the qualitative test were zero. The bottom line for this program is: the technology evaluated rendered orders of magnitude greater number of spores nonviable than required. A recommendation for future testing is adjusting the coupon size such that the true ORD inoculation and ORD comparison can be directly made.

LITERATURE CITED

1. Jahnke, M.; Lauth, G. *Biodecontamination of a Large Volume Filling Room with Hydrogen Peroxide*. Pharm. Eng. **1997**, pp 2-12.
2. McDonnell, G.G.; Gringol, G.; Antloga, K. *Vapor Phase Hydrogen Peroxide Decontamination of Food Contact Surfaces*. Dairy, Food Environ. Sanit. **2002**, pp 868-873.
3. Wagner, G.; Sorrick, D.; Procell, L.; Brickhouse, M.D.; McVey, I.; Schwartz, L. *Decontamination of VX, GD, and HD on a Surface Using Modified Vaporized Hydrogen Peroxide*. Langmuir **2007**, 23, pp 1178-1186..
4. Brickhouse, M.D.; Lalain, T.; MacIver, B.; Pfarr, J.; Procell, L.; Schultz, M.; Sorrick, D.; Turetsky, A.; Wagner, G.; Schwartz, L.; McVey, I.; Meilander, T.; Wiget, P. *The Development of Modified Vaporous Hydrogen Peroxide (mVHP) for Chemical and Biological Weapons Decontamination*. Presented at the Decon Conference, Tuscon, AZ, 2005.
5. Brickhouse, M.D.; Turetsky, A.; MacIver, B.; Pfarr, J.; McVey, I.; Alter, W.; Lloyd, J.; Fonti, M. *Vaporous Hydrogen Peroxide Decontamination of a C-141B Starlifter Aircraft Validation of VHP and mVHP Fumigation Decontamination Process via VHP-Sensor, Biological Indicator, and HD Simulant in a Large-Scale Environment*; ECBC-TR-510; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2007.
6. Brickhouse, M.D.; Turetsky, A.; McVey, I. *Decontamination of CBW Agents by mVHP: Demonstration of the CBW Decontamination of a Building using mVHP*; ECBC-TR-470; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2007.
7. Brickhouse, M.D.; Lalain, T.; Pfarr, J.; MacIver, B.; Lloyd, J.; Flowers, J.; Mantooth, B.; Zander, Z.; Stark, D.; Shue, M.; Dutt, D. *Validation of FMTV Modular VHP/mVHP System and Fumigation Decontamination Process of a C-141B Starlifter Aircraft*; ECBC-TR-522; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2007.
8. Lalain, T.; Brickhouse, M.D.; Gater, S.; Williams, K.; Hendershot, J.; Rodriguez, A.; Mantooth, B.; Stark, D. *Chemical-Warfare Agent Decontamination Efficacy Testing: Large-Scale Chamber mVHP Decontamination System Evaluation. Part I: Comparison to ORD*; ECBC-TR-527; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2007.
9. Lalain, T.; Brickhouse, M.D.; Pfarr, J.; Lloyd, J.; Flowers, J.; Mantooth, B.; Zander, Z.; Stark, D. *Evaluation of the Steris Sensitive Equipment Decontamination (SED) Apparatus on a 463L Pallet*; ECBC-TR-526; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2007

10. JRO *Joint Platform Interior Decontamination (JPID) Operational Requirements Document (ORD)*, 2005.

11. JRO *Operational Requirements Document for Joint Service Sensitive Equipment Decontamination*, 2003.

12. McVey, I.; Schultz, M.; Centanni, M.; McDonnel, G. Activated Oxidizing Vapor Treatment System and Method. *USPTO App. U.S. 2004/0057868 A1*: United States, 2004.

13. McVey, I.; Schwartz, L.; Centanni, M.; Wagner, G. Activated Vapor Treatment for Neutralizing Warfare Agents. *USPTO App. U.S. 2004/0215046 A1*: United States, 2004.

14. Brickhouse, M.D.; Lalain, T.; MacIver, B.; Procell, L.; Schultz, M.; Sorrick, D.; Turetsky, A.; Pfarr, J.; Wagner, G.; Schwartz, L.; McVey, I.; Meilander, T.; Wiget, P. The Development of Modified Vaporous Hydrogen Peroxide (mVHP) for Chemical and Biological Weapons Decontamination. In *CB Defense Conference*; Timonium, MD, 2005.

GLOSSARY

APG	Aberdeen Proving Grounds
BI	biological indicator
BSL-3	biosafety level three
BW	biological warfare
CARC	Chemical Agent Resistant Coating
CB	chemical and biological
cfm	cubic feet minute
CFU	colony forming unit
CofA	certificate of analysis
CRADA	Cooperative Research and Development Agreement
CT	concentration time
CW	chemical warfare
DoD	Department of Defense
DS	Decontamination Sciences
ECBC	Edgewood Chemical Biological Center
H ₂ O ₂	hydrogen peroxide
GD	nerve agent, soman
<i>G. stearo</i>	Shorthand for biological surrogate <i>G. stearothermophilus</i>
HD	blister agent, mustard
hr or hrs	hour or hours
IAW	in accordance with
IOP	Internal Operating Procedure
JPID	Joint Platform Interior Decontamination
JSSD	Joint Service Sensitive Equipment Decontamination
KPP	Key Performance Parameters
LOE	Limited-Objective Experiment
min	minutes
MSDS	Material Safety Data Sheets
mVHP®, mVHP	reference to Steris' registered "modified vaporized hydrogen peroxide" procedure
ORD	Operational Requirements Document
PI	principal investigator
PPE	personal protective equipment
ppm	parts per million
Pre-Op	pre-operational
psi	pounds per square inch
R&D	Research and Development
RDECOM	Research, Development, and Engineering Command (formerly SBCCOM)
RH	relative humidity
RRO	Risk Reduction Office
SBCCOM	Soldier and Biological Chemical Command
SD	standard deviation

SED	sensitive equipment decontamination
SOPs	standing operating procedures (standard may also be used in place of standing with the same meaning)
T	temperature
t	time
TGD	nerve agent, thickened soman
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
SOR	start of run
TWA	time-weighted average
VHP [®] , VHP	reference to Steris' registered "vaporized hydrogen peroxide" procedure
VX	nerve agent

APPENDIX A

COUPON STOCK MATERIAL AND PREPARATION

Glass

- Type: Heat-Resistant Borosilicate Glass
- Supplier: McMaster-Carr
- Stock Material: individual 13-mm diameter by 3-mm thick heat-resistant borosilicate sight glasses

Aluminum

- Type: 5052
- Supplier: E-J Enterprises
- Stock Material: received as 48" x 120" sheets, 0.125" thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop.

Chemical Agent Resistant Coating (CARC)-painted Aluminum

- Type: Aluminum 5052, painted with Forest Green CARC, MIL-C-53039A
- Supplier: E-J Enterprises
- Stock Material: received as 48" x 120" sheets, 0.125" thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop then painted on one face plus edges with Chemical Agent Resistant Coating, MIL-C-53039A, according to established procedures.

Polycarbonate

- Type: Clear Polycarbonate Sheet
- Supplier: E-J Enterprises, order # 0001-03460
- Stock Material: received as 48" x 96" sheets, 0.22" thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop.

US Air Force Topcoat Painted Aluminum

- Type: Aluminum 5052, painted with Grey USAF Topcoat, MILK-PRF-85285
- Supplier: E-J Enterprises
- Stock Material: received as 48" x 120" sheets, 0.125" thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop then painted on one face plus edges with US Air Force Topcoat, MILK-PRF-85285.

Silicone Elastomer

- Type: Silicone Elastomer - Sheet MQ/VNQ/PMQ/PVMQ
- Supplier: Goodfellow, Order #089-628-36
- Stock Material: received as 500 mm x 500 mm sheets, 3.0 mm thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop.

Kapton®

- Type: Polyimide (PI) Film, grade Kapton HN
- Supplier: Goodfellow, order # LS257291
- Stock Material: received as 610 mm x 2 m coil, 0.125 mm thick
- Preparation Details:
 - o Biological surrogate tests: 1.3-cm squares cut from stock material at ECBC Fabrication shop.

Viton® (Gasket Material, n-nitrile)

- Type: Hexafluoropropylene-vinylidene fluoride copolymer sheet FKM
- Supplier: Goodfellow, order # FV313300
- Stock Material: received as 300 mm x 300 mm sheets, 3.0 mm thick
- Preparation Details:

APPENDIX B

MVHP CONTROL CHARTS

B.1 SCOPING TEST (RUN B1)

Figure B.1.1: Concentration Control Chart

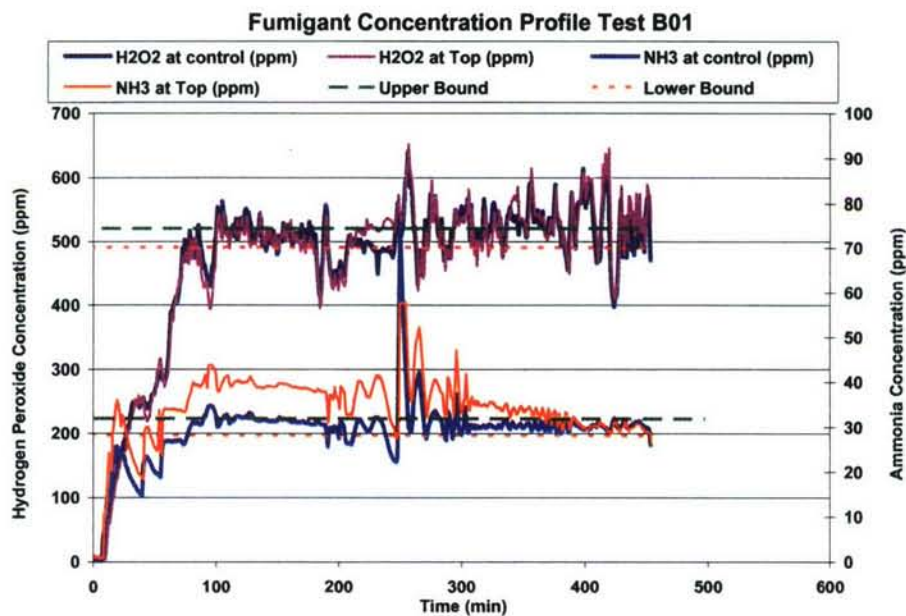


Figure B.1.2: Temperature and Humidity Control Chart

Temperature (C) & Percent Relative Humidity
for Test B01

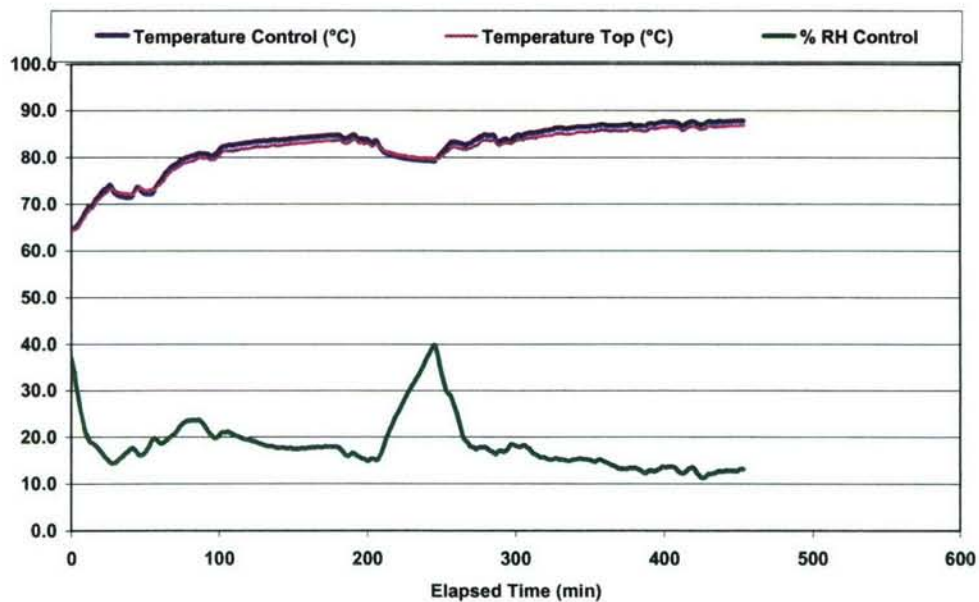


Figure B.2.1: Concentration Control Chart

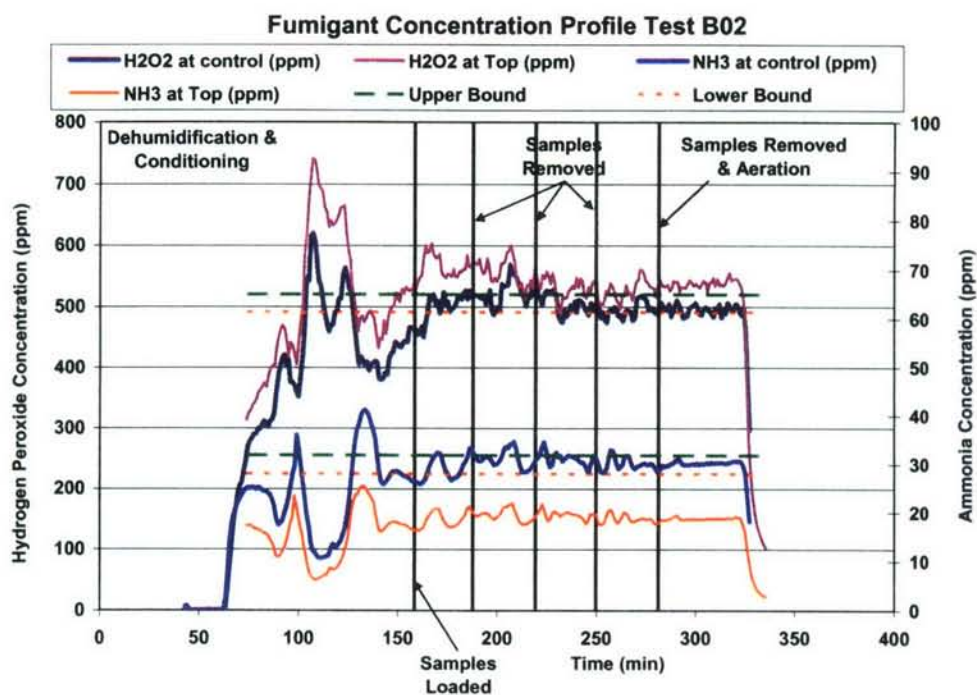


Figure B.2.2: Temperature and Humidity Control Chart

**Temperature (C) & Percent Relative Humidity
for Test B02**

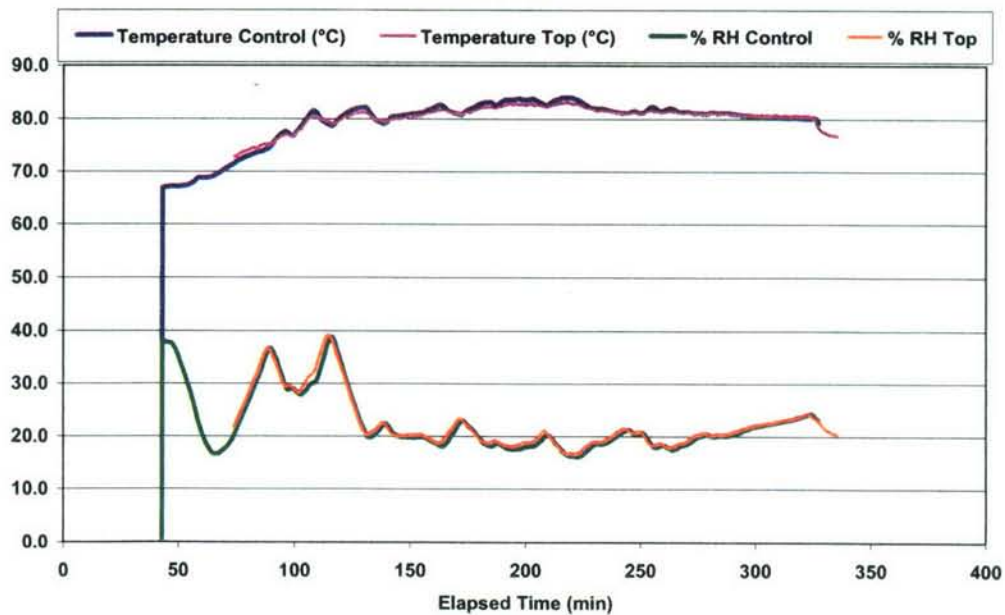


Figure B.3.1: Concentration Control Chart

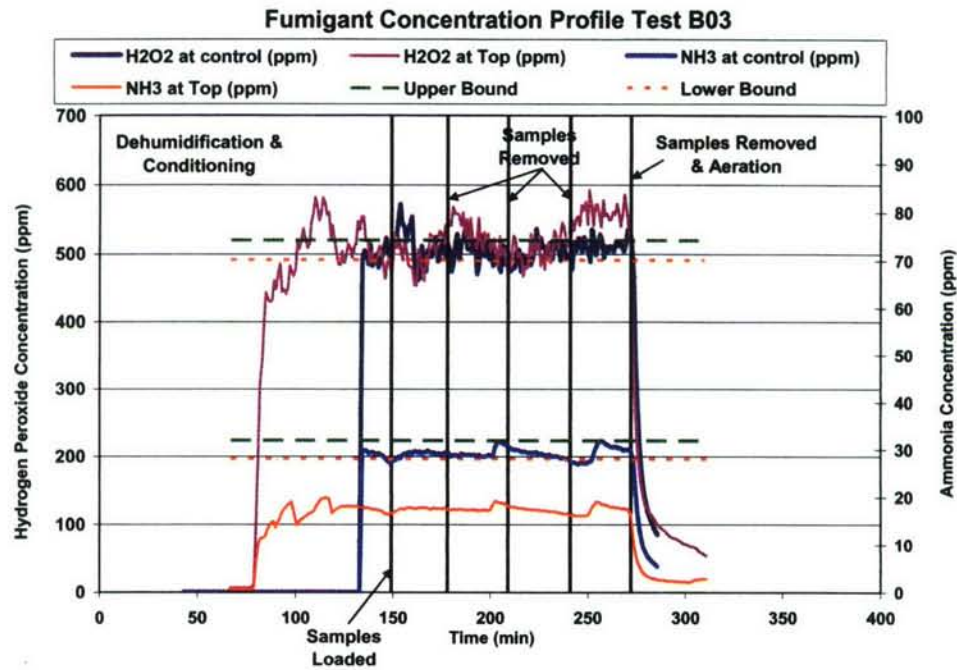


Figure B.3.2: Temperature and Humidity Control Chart

Temperature (C) & Percent Relative Humidity
for Test B03

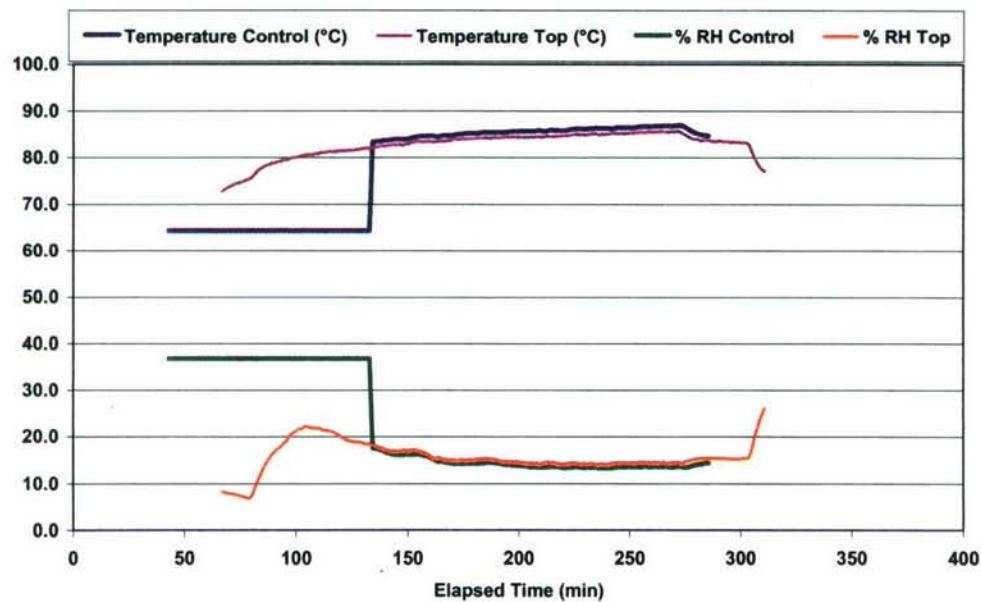


Figure B.4.1: Concentration Control Chart

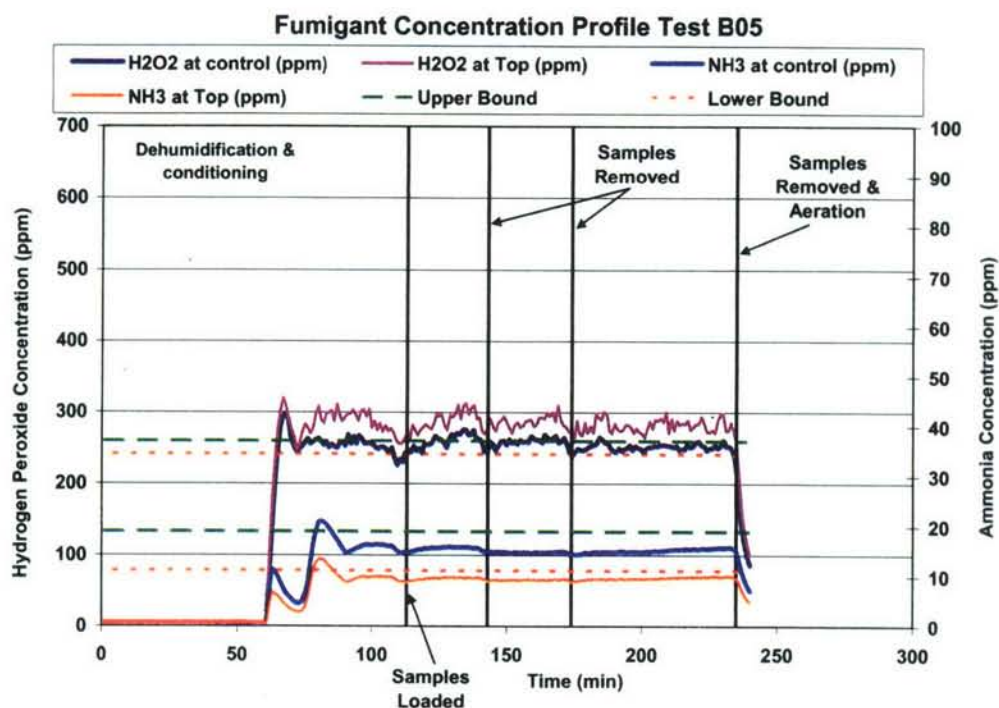


Figure B.4.2: Temperature and Humidity Control Chart

**Temperature (C) & Percent Relative Humidity
for Test B05**

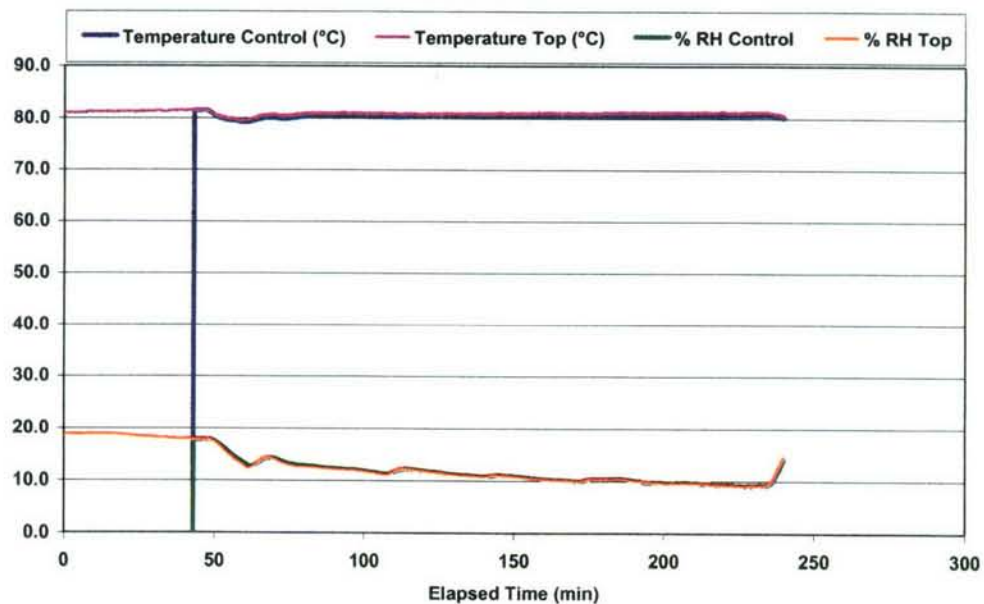


Figure B.5.1: Concentration Control Chart

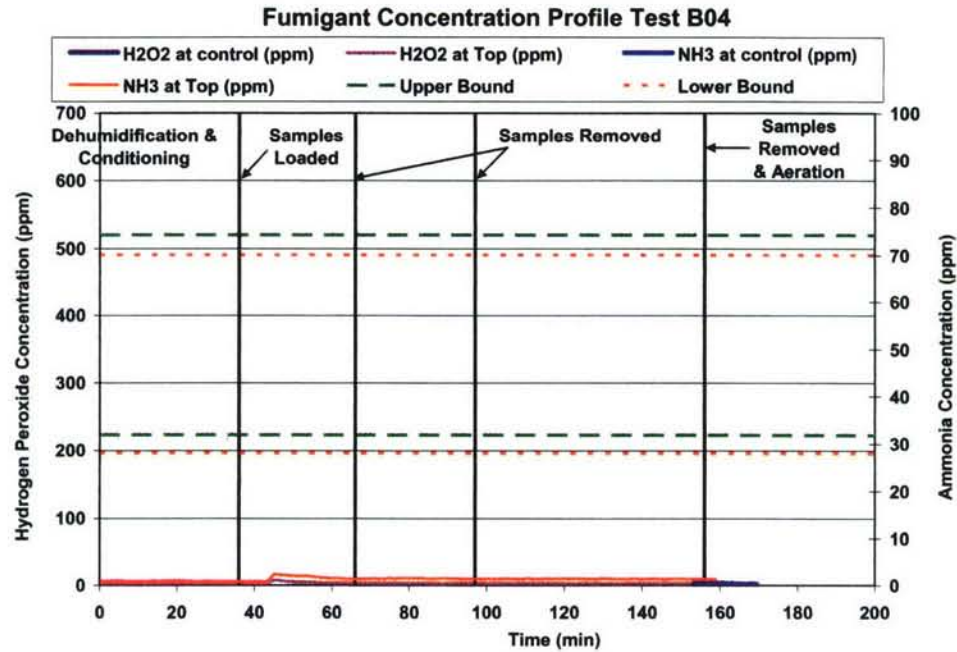


Figure B.5.2: Temperature and Humidity Control Chart

Temperature (C) & Percent Relative Humidity
for Test B04

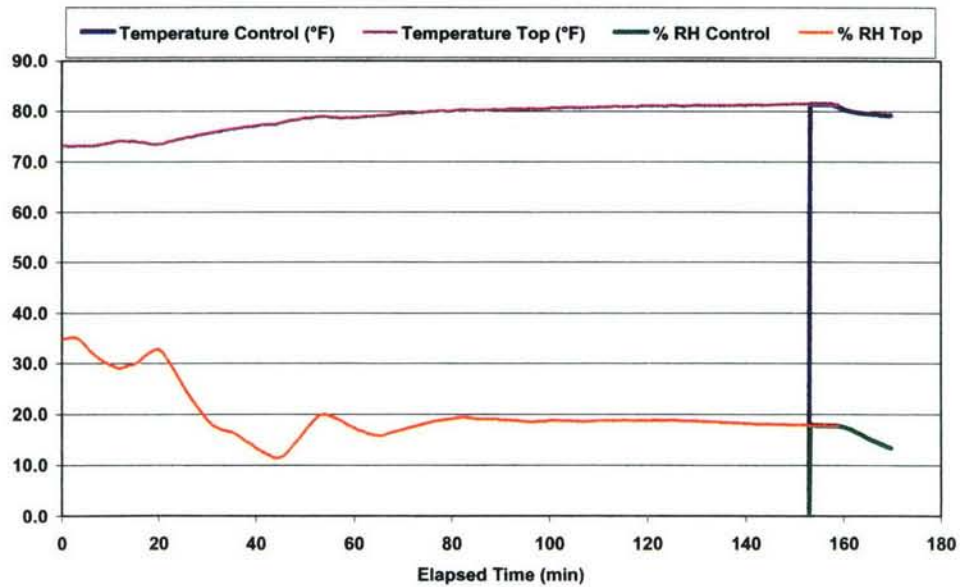


Figure B.6.1: Concentration Control Chart

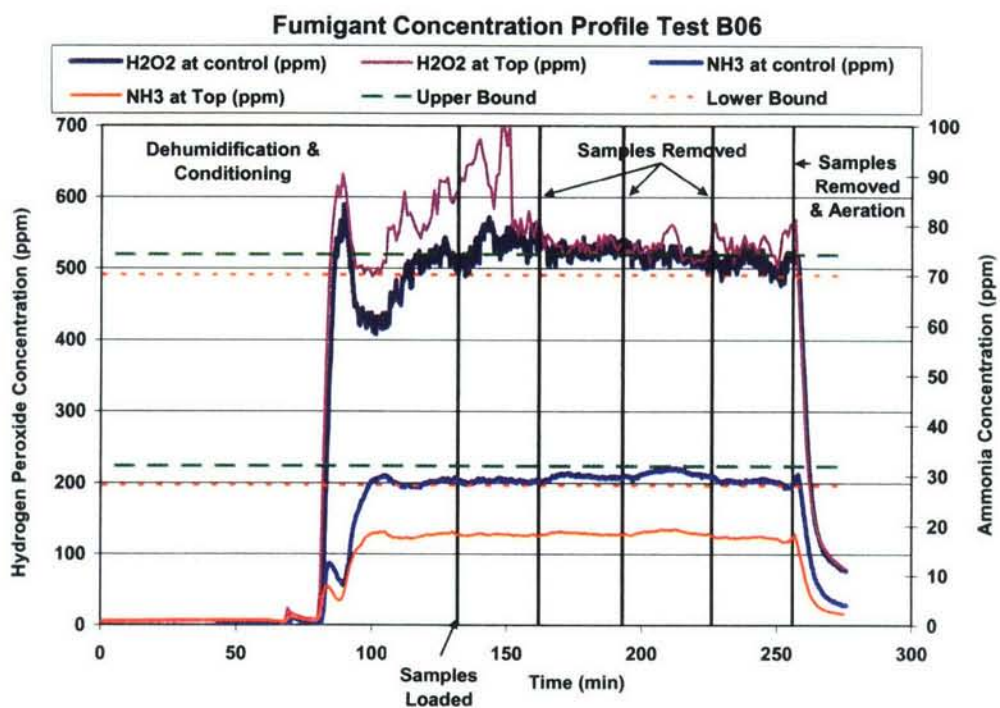


Figure B.6.2: Temperature and Humidity Control Chart

**Temperature (C) & Percent Relative Humidity
for Test B06**

