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# Vaporized Hydrogen Peroxide (VHP<sup>®</sup>) Decontamination of a Section of a Boeing 747 Cabin

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16. Abstract  The use of STERIS Corporation's Vaporized Hydrogen Peroxide (VHP <sup>®</sup> )* technology as a potential biocide for aircraft decontamination was demonstrated in a cabin section of the Aircraft Environment Research Facility (an FAA-owned Boeing 747). When exposed to an appropriate concentration of VHP vapor in the cabin test section, biological indicators inoculated with 10 <sup>6</sup> colony forming units of <i>Geobacillus stearothermophilus</i> spores demonstrated a total suppression of culture growth. Efficacy was demonstrated with and without seats in the test section of the aircraft. The importance of adequate air mixing was also demonstrated.  *VHP is a registered trademark of the STERIS Corporation					
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# VAPORIZED HYDROGEN PEROXIDE (VHP®) DECONTAMINATION OF A SECTION OF A BOEING 747 CABIN

## INTRODUCTION

Strategic Technology Enterprises (STE) a subsidiary of STERIS Corporation, and the Federal Aviation Administration (FAA) Civil Aerospace Medical Institute (CAMI) established a Cooperative Research and Development Agreement (CRDA) to evaluate Vaporized Hydrogen Peroxide (VHP®) technology as a potential aircraft decontaminant and to utilize the distribution pattern of the VHP vapor to support validation of theoretical cabin air flow models.

Under this CRADA, Strategic Technology Enterprises would support CAMI in the validation of a Computational Fluid Dynamics (CFD) model for aircraft cabins. It was anticipated that the ability to accurately measure VHP distribution patterns would assist in confirming CFD model predictions. The use of VHP technology could provide information relative to aircraft decontamination as well as the distribution of decontaminant materials.

STE supplied the VHP technology, real time monitoring capability (near-infrared spectrophotometers), and personnel to operate the STE-supplied equipment. CAMI supplied the test facility, support personnel, and made the operational modifications needed to conduct the work. The equipment was set up and operated in a passenger section of the CAMI Boeing 747 Aircraft Environment Research Facility (AERF) cabin. A series of short trials were performed to develop the data necessary to verify the CFD model. Following the model validation trials, STE conducted trial runs in the same cabin section of the AERF using biological and chemical indicators (BIs and CIs) to provide data relative to the use of VHP vapor as a decontaminant. This report details the portions of the CRADA that pertain to the BI runs performed in the FAA/CAMI 747. Work related solely to the CFD verification support will not be addressed in this document unless it is relevant to the BI/CI runs.

## BACKGROUND

Six years ago, CAMI obtained a Boeing 747-100 fuselage to customize for use in studies and training to promote and improve aircraft cabin safety. The plane was designated the AERF. A project initiated in 2000 utilized the AERF as a test bed for the development of advanced

simulations based on CFD to study contaminant distribution aboard transport aircraft. The development of CFD models using the 747 AERF as the realistic test and validation facility is an ongoing effort with model development by the University of Tennessee - Computational Fluid Dynamics Laboratory (UT-CFDL) and validation support by the CAMI Environmental Physiology Research Team. Although the CFD approach is applicable to a variety of cabin air quality issues, the focal aspect of these studies has been the distribution characteristics associated with the release of a chemical or biological agent in flight. Since this environment has been characterized in terms of airflow and used to support development of predictive algorithms, it represented a realistic environment for studying potential means of decontamination from a noxious biological exposure whether intentionally introduced into the cabin or inadvertently carried aboard through passenger illness.

The STERIS Corporation developed the VHP process, which has been widely used for the routine decontamination of enclosed environments and surfaces (1). It is an environmentally friendly technology and is in widespread use for sterile pharmaceutical processing (2), sterility testing (3), lyophilizer decontamination (4), biosafety cabinet decontamination, animal laboratory decontamination (5), equipment decontamination, etc. The biocide has been shown to be sporicidal (6), tuberculocidal (7), virucidal (8), bactericidal (9,10), fungicidal (9,10), and has been successfully used for the bioremediation of buildings contaminated with highly resistant biological weapons such as *Bacillus anthracis* spores. VHP technology demonstrates an excellent material compatibility profile, including use on electronic and electrical equipment (5,8), which is a highly desirable characteristic for use in aircraft interior decontamination.

Recognizing that the possibility of cabin contamination exists, a validated, reliable means of decontaminating the aircraft and returning it to service is in the interest of passengers, crew, and aircraft operators. Developing decontamination strategies requires very meticulous investigation and analysis since the passenger cabin represents very specific environmental and performance constructs that cannot be compromised. Thus, the objective of this project was to demonstrate the effectiveness of VHP technology in eradicating representative biological contamination in a commercial transport environment and to use

the VHP distribution patterns to assist in the validation of the CFD models developed by UT-CFDL.

## METHODS AND MATERIALS

Testing consisted of placing chemical and biological indicators in a section of the AERF Boeing 747-100 aircraft. The VHP process was then introduced into the cabin section using four VHP 1000 generators. The VHP concentration and effectiveness were measured through the use of biological indicator coupons and VHP sensors.

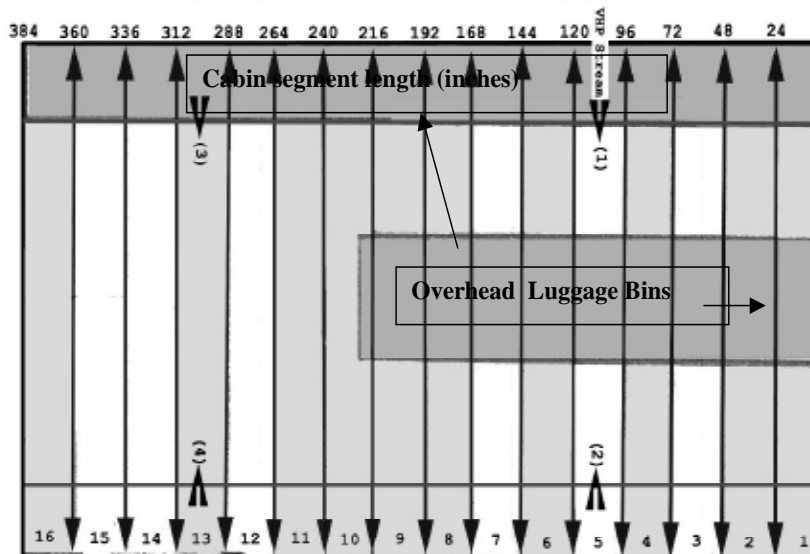
*Test Facility:* The AERF at the FAA/CAMI was utilized as the test facility. For these tests, a passenger cabin segment just aft of the entrance door in front of the wing was utilized. The segment is 32 feet long with a volume of approximately 5000 cubic feet. The test section was separated from the rest of the cabin by two bulkheads built specifically for the purpose of segmenting sections of the AERF. Tape and plastic sheeting was used throughout the cabin to minimize VHP leakage during the testing. The test section is shown schematically in Figure 1.

*VHP Generation:* The VHP process was introduced into the cabin at four locations. Two generators (STERIS VHP 1000) were each located at either end of the cabin segment, outside of the test area itself. The generators are completely self-contained bio-decontamination systems with the ability to dehumidify, generate VHP vapor, and aerate sealed enclosures. The inlets and outlets of the generators passed into the cabin segment through openings in the bulkheads. Figure 1 also shows where the

inlet points were located. The back bulkhead was used as the zero reference point of the Z-axis of the Cartesian grid characterizing the cabin segment. The generator inlet tubes were located at 108 and 216 inches from the back bulkhead on either side of the cabin. The inlets were 76 inches off the floor (Y-axis), the outlet being located even with the bottom of the overhead luggage bin (Figure 2). In a typical run, the generators were set to run an 80- minute cycle, during which a 35% solution of VHP sterilant (Vaprox®)\* was injected at a rate of 12 grams per minute. Therefore, the total amount of Vaprox injected into the cabin segment during a run was approximately 3.88 kg, of which 1.36 kg was hydrogen peroxide vapor and 2.52 kg was water vapor.

*VHP Process Quantification Methods:* Two methods were used to quantify the VHP concentrations in the cabin test section during the experiments. Near-infrared spectrophotometers (Hydrogen Peroxide Vapor Monitor, Guided Wave Inc., El Dorado Hills, CA) provided a real-time measurement of the VHP concentration in the range of 0.1 to 50 mg/l. The second VHP concentration measurement was accomplished using Chemical Indicators, (Steris Corp., 5960 Heisley Rd, Mentor, OH), which provided a simple, non-quantified color change indication that VHP vapor was present at the indicator location. CIs were paired with biological indicators, as described below. In addition, an estimate of VHP vapor delivery into the cabin could be derived from the velocity of the flow stream and the cross sectional area of the inlet tube. Hot wire anemometers were placed in the middle of the VHP inlet and the velocity recorded. The resulting

**Figure 1.** Diagram of the 747 AERF cabin section used to evaluate cabin airflow and VHP distribution. VHP vapor introduction points are identified in sections 5 and 13.



\* Vaprox is a registered trademark of the STERIS Corporation

**Figure 2.** Photograph showing an overhead luggage rack, VHP vapor introduction piping, and hotwire anemometer.



**Figure 3.** Photograph 1 showing the bulkhead penetration of the VHP Inlet pipe and VHP Return (open connector) into the test section of the AERF. Photograph 2 showing the STERIS model 1000 VHP.



Photograph 1.

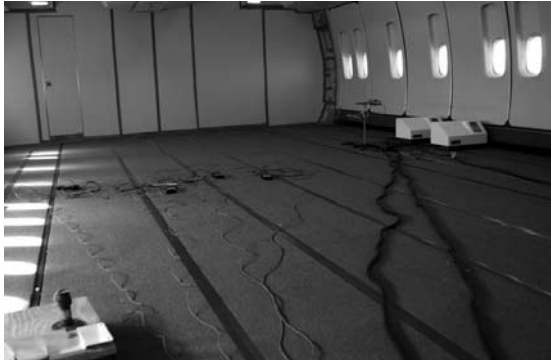


Photograph 2.

**Figure 4.** Photographs showing the 747 AERF section with the seats removed and bulkheads isolating the test section of the aircraft. Photograph 1 is a view of the Forward bulkhead, and Photograph 2 is a view of the Aft bulkhead.



Photograph 1.



Photograph 2.

**Figure 5.** Photograph 1 shows the Guided Wave Hydrogen Peroxide monitors installed in the test section of the 747 AERF. Photograph 2 illustrates how the Guided Wave and hot wire anemometer data cables were routed through the floor seat tracks.



Photograph 1.



Photograph 2.

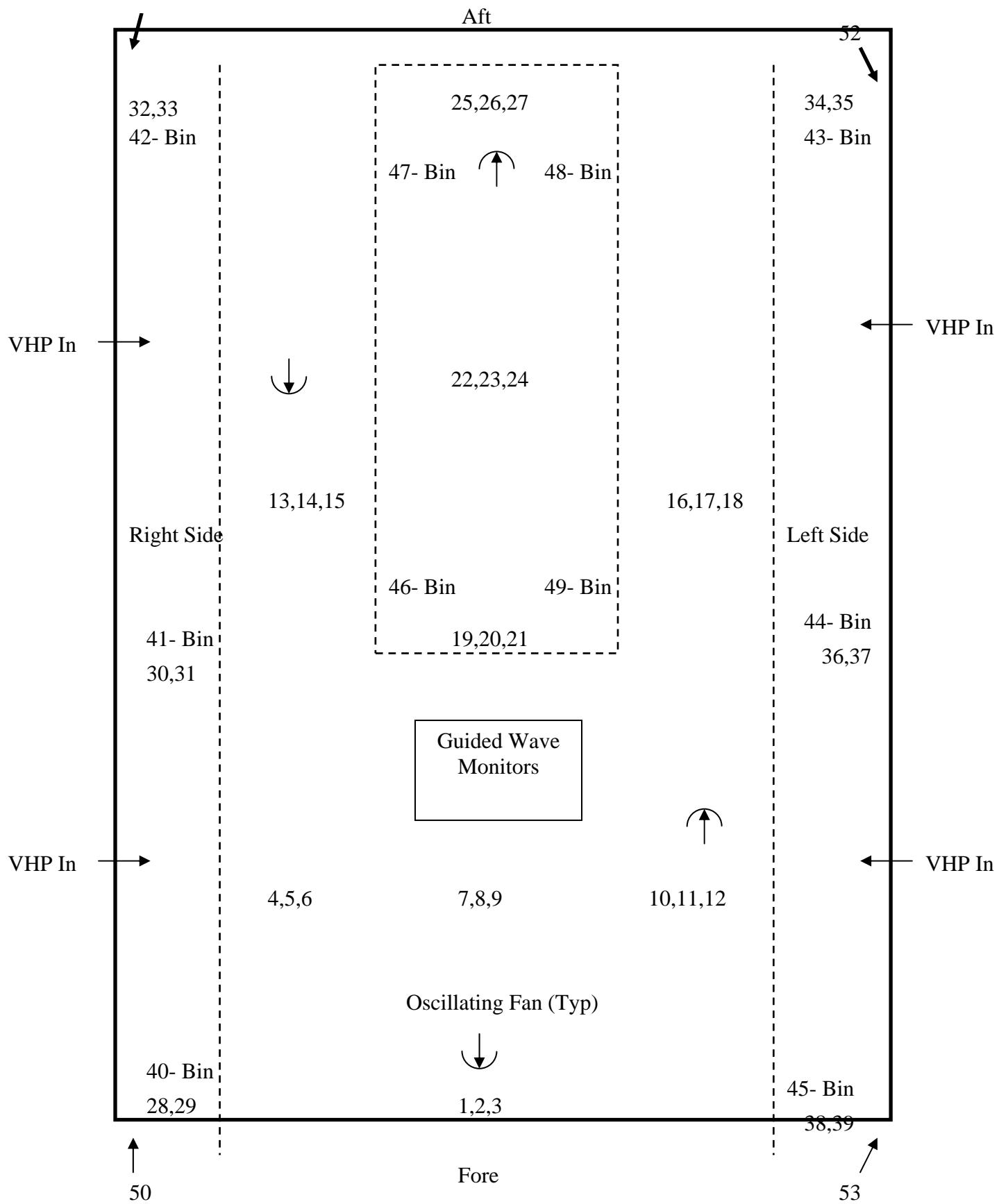


**Table I.** Identification and location of BI and CI test strips as shown in Figure 5.

<b>Sensor No. / Location</b>	<b>Sensor No./ Location</b>
1 Fore Bulkhead, Center, 88" From Floor	29 Under Right Luggage Area, Fore, Corner, Low
2 Fore Bulkhead, Center, 56" From Floor	30 Under Right Luggage Area, Mid Cabin Wall, High
3 Fore Bulkhead, Center, 24" From Floor	31 Under Right Luggage Area, Mid Cabin Wall, Low
4 Right Fore Cabin, 88" From Floor	32 Under Right Luggage Area, Aft, Corner, High
5 Right Fore Cabin, 56" From Floor	33 Under Right Luggage Area, Aft, Corner, Low
6 Right Fore Cabin, 24" From Floor	34 Under Left Luggage Area, Aft, Corner, High
7 Center Fore Cabin, 88" From Floor	35 Under Left Luggage Area, Aft, Corner, Low
8 Center Fore Cabin, 56" From Floor	36 Under Left Luggage Area, Mid Cabin Wall, High
9 Center Fore Cabin, 24" From Floor	37 Under Left Luggage Area, Mid Cabin Wall, Low
10 Left Fore Cabin, 88" From Floor	38 Under Left Luggage Area, Fore, Corner, High
11 Left Fore Cabin, 56" From Floor	39 Under Left Luggage Area, Fore, Corner, Low
12 Left Fore Cabin, 24" From Floor	40 Right Luggage Bin, Fore
13 Right Mid Cabin, 88" From Floor	41 Right Luggage Bin, Mid Cabin
14 Right Mid Cabin, 56" From Floor	42 Right Luggage Bin, Aft
15 Right Mid Cabin, 24" From Floor	43 Left Luggage Bin, Aft
16 Left Mid Cabin, 88" From Floor	44 Left Luggage Bin, Mid Cabin
17 Left Mid Cabin, 56" From Floor	45 Left Luggage Bin, Fore
18 Left Mid Cabin, 24" From Floor	46 Center Luggage Bin, Right Side, Mid Cabin
19 Under Center Luggage Area, Fore End, 72" From Floor	47 Center Luggage Bin, Right Side, Aft
20 Under Center Luggage Area, Fore End, 56" From Floor	48 Center Luggage Bin, Left Side, Aft
21 Under Center Luggage Area, Fore End, 24" From Floor	49 Center Luggage Bin, Left Side, Mid Cabin
22 Under Center Luggage Area, Middle, 72" From Floor	50 Top Right Corner, Fore (Above Luggage Bin)
23 Under Center Luggage Area, Middle, 56" From Floor	51 Top Right Corner, Aft (Above Luggage Bin)
24 Under Center Luggage Area, Middle, 24" From Floor	52 Top Left Corner, Aft (Above Luggage Bin)
25 Under Center Luggage Area, Aft End, 72" From Floor	53 Top Left Corner, Fore (Above Luggage Bin)
26 Under Center Luggage Area, Aft End, 56" From Floor	
27 Under Center Luggage Area, Aft End, 24" From Floor	
28 Under Right Luggage Area, Fore, Corner, High	

**Figure 6.** Photograph of BIs and CIs (positions no. 25 – 27, as shown in Figure 7 and identified in Table I) attached to wire in preparation for testing.





**Figure 7.** Cabin section showing location of monitors, VHP inlets, and circulating fans.

volume was then converted to a VHP mass estimate. Dräger Accuro hand pump environmental monitors, with 0.1 to 3.0 ppm H<sub>2</sub>O<sub>2</sub> sensitivity detection tubes, were used to monitor potential VHP leakage from the test area and to verify interior VHP residual levels at the end of a test.

**Biological Indicators (BIs):** BIs were used to provide functional assessments of the VHP level present in the cabin. The biological indicators were tri-pack stainless steel coupons inoculated with approximately 10<sup>4</sup>, 10<sup>5</sup>, and 10<sup>6</sup> colony forming units (CFU) with *Geobacillus stearothermophilus* spores packaged in sub-divided Tyvek<sup>®\*</sup> envelopes (Apex Laboratories, Inc.). The CI and BI packets were distributed throughout the cabin segment using wires with paper clips located at positions to give the desired height for each BI/CI set. The CI/BI locations were numbered sequentially (1 – 53) and located in the cabin segment as shown in Figure 7. In addition to the BIs exposed during the testing, two indicators that were not exposed to VHP treatment were cultured as a positive growth control. Following each test the BIs were left sealed in their Tyvek envelopes, packaged separately from the controls, and transported to STERIS laboratories for analysis.

**Biological Indicator Processing:** Each BI consisted of three metal coupons. One coupon was inoculated with 10<sup>4</sup> CFU, one with 10<sup>5</sup> CFU, and one with 10<sup>6</sup> CFU. Tryptic soy broth was aseptically dispensed into sterile test tubes in 10 ml aliquots. The Tyvek envelopes containing the BI coupons were aseptically opened in a biological safety cabinet, the coupons removed from the envelope with sterile forceps, and each coupon was placed in an individual tube of media. The tubes were incubated at 57°C for 24 hours prior to being scored for growth/no growth. The positive controls were transferred into the media last to avoid cross-contamination. After scoring for growth/no growth, tubes were selected for Gram staining to confirm the identity of any growing organism(s). If one tube in a series was negative for growth, another tube from the same series was selected for Gram staining. If

all the tubes in one rack were positive for growth, a tube was randomly selected for Gram staining.

**Test Conditions:** Table II shows the test conditions used during the VHP technology evaluations. To develop a basic understanding of the airflow pattern and distribution of the VHP process, the first four tests were conducted with the passenger seats removed. The passenger seats were reinstalled in the cabin section for the remaining two tests. The first test was conducted to evaluate the cabin air circulation patterns; therefore, the cabin air circulation was in operation. The objectives of the remaining tests were to assess the distribution and effectiveness of the VHP generators with/without auxiliary circulation, so the aircraft air circulation system was not used. The VHP generators had the capability to recirculate air from the test section of the cabin, and this recirculation feature was evaluated in test series 2, 3, and 5. Four standard 16 in. oscillating fans were used to assist air mixing in tests 3-6.

**Cabin Air Circulation On** represents only the use of the 747's built-in air circulation system. This system feeds air to the cabin just below the outboard luggage bins and removes air via the outboard floor level vents.

**VHP Recirculation Yes** incorporated the VHP 1000's built-in capability to recirculate hydrogen peroxide laden air from the treated space through the generator where it was freed from residual hydrogen peroxide and water vapor, then recharged with VHP vapor prior to being reintroduced to the treated space.

**VHP Recirculation No** utilized the VHP 1000 only to feed VHP laden air into the treated space; the spent hydrogen peroxide vapor was removed from the cabin test section via the outboard floor vents, driven by a very slight overpressure effect.

Note: Pre-test equipment evaluations determined that the 1¼" piping installed for the VHP inlet generated a high airflow velocity relative to the cabin flow and would disrupt the cabin air circulation patterns for CFD model support work. To reduce the VHP inlet flow velocity, a 3" diameter inlet pipe was installed on the one VHP

TABLE II. Test Matrix and description of test conditions.

Test Series	Cabin Configuration	Cabin Air Circulation	VHP Recirculation	Auxiliary Oscillating Fans
1	No seats	On	No	No
2	No seats	Off	Yes	No
3	No seats	Off	Yes	Yes
4	No seats	Off	No	Yes
5	Seats Installed	Off	Yes	Yes
6	Seats Installed	Off	No	Yes

\* Tyvek is a registered trademark of the DuPont Corporation.

1000 generator inlet pipe that would be used for the CFD work. With the expected flow velocity reduction, the new inlet installation was insulated to ensure that VHP condensation did not occur as the flow was slowed in the pipe. Subsequent testing confirmed the calculated velocity reduction. Upon completion to these first (CFD) tests, the VHP inlet from the generator was returned to its original configuration for use in the BI/CI tests.

## RESULTS AND DISCUSSION

The biological indicator results from all runs are shown in Table III. A reading of  $<10^4$  may indicate minimal biological reduction, while a reading of  $>10^6$  indicates complete kill of all coupons contained on the particular biological indicator. Intermediate levels of biological reduction are reported based on the coupon showing no growth, where growth was seen on the next higher-level coupon.

Test 1 results, with only the aircraft ventilation system running for VHP distribution, showed measurable kill only in the biological indicators that were in a nearly direct line with the VHP inlets. The VHP-affected BIs were located under the central luggage bins at the intermediate and low levels. This suggests that in the absence of auxiliary air mixing there is minimal VHP migration in the fore-aft direction within the cabin, and that the VHP vapor was highly diluted by incoming ventilation air and efficiently removed by the floor level vents before a sporicidal concentration could be reached. It should be noted that the AERF ventilation system was incapable of being run with full or partial recirculation; it could only be run as a flow-through system. An average VHP concentration is not meaningful for this run where a non-uniform concentration was observed.

Test 2 was performed to determine mixing effectiveness with the four auxiliary fans in use. The run was initiated and then the four VHP 1000 generators sequentially aborted at times of 3, 4, 8.5 and 34 minutes into their VHP cycles. The results of the biological indicators show that a very consistent level of kill (5 log) was experienced. The measured maximum VHP concentration when the fourth generator was aborted was 285 ppm. The consistency of kill level demonstrates that the atmosphere within the test section was efficiently mixed by the four oscillating fans supporting the uniform level of kill observed.

Tests 3 and 4 were designed to determine if there was any difference between running the VHP generators in the normal recirculation mode (as used in the majority of room and isolator applications) versus allowing spent

hydrogen peroxide vapor to exit the test section via the cabin exhaust ventilation system. These two runs were completed without the cabin seats in place, so the potential perturbation to the airflows that the seats might cause would not be a factor. Under the test conditions, both techniques demonstrated complete kill, and both runs showed average maximum VHP concentrations of approximately 600 ppm.

Tests 5 and 6 were a repeat of the conditions of runs 3 and 4, respectively. For these tests, all of the cabin area seats were replaced to provide a situation more consistent with that expected in a real-world decontamination event (no pictures of the cabin set up as run with seats are available; the normal 3-5-3 layout for each row of seats was used). The possibility still existed that the presence of the seats would have an impact on VHP distribution with only the four auxiliary fans to provide mixing. The results were clear, and both runs demonstrated complete kill and average maximum VHP concentrations comparable to tests 3 and 4.

The CIs used in this evaluation provided a non-qualitative indication of the presence or absence of VHP vapor. During the first test (AERF air circulation operating), only the CIs located in close proximity to the VHP outlets showed a color change indicating the presence of VHP vapor. CI results from the second test (in which the VHP generators were sequentially shut down) showed uniform partial color change, indicating a moderate and uniform concentration of VHP vapor throughout the cabin. CIs in all subsequent tests showed a complete color change, indicating a high concentration of VHP vapor. During Test 1, the Guided Wave monitors were located near the centerline of the aircraft section on the same fore-aft cabin axis as the VHP generator inlet. On the vertical axis, one of the VHP sampling points was located high, just under the center luggage bin, and the other was located halfway between the floor and the luggage bin. The monitor located high indicated a maximum VHP concentration of 213 ppm. The mid-height sensor indicated a maximum VHP concentration of 780 ppm. The BIs located in this area (locations 17, 18, and 23) showed a partial degree of sporicidal action, indicating a localized concentration of VHP vapor. Test 2, with non-uniformly functioning VHP generators, showed an even distribution of VHP vapor, albeit at a relatively low concentration (maximum 285 ppm). The VHP concentration in the remaining tests averaged 550 – 640 ppm and, as indicated by the maximum color change of the CIs and uniform sporicidal action measured by the BIs, was uniformly distributed throughout the cabin section.



## CONCLUSIONS

When adequate concentrations of VHP vapor were developed in the test section of the CAMI 747AERF, a complete kill of test coupons inoculated with  $10^6$  CFU of *Geobacillus stearothermophilus*, a spore-forming microorganism, was achieved. Four STERIS VHP 1000 generators running at a Vaprox injection rate of 12g/min provided an adequate sporicidal concentration of VHP vapor for exposed areas of the 5000 cubic foot aircraft section. When fans were operated to support thorough air mixing, this level of sporicidal action was demonstrated uniformly throughout the test cabin with and without passenger seats installed. The complete organism kill was achieved on test coupons located in open-air areas of the cabin and on test coupons located in the luggage bins. Evaluations of the effect of VHP vapor on materials were not conducted, and the sporicidal effect of VHP vapor on carpets, seat cushions, and less accessible areas of the aircraft was not evaluated. However, no observable changes to the materials of the cabin test section or its contents were noted at the conclusion of the VHP live testing. When only the AERF cabin air ventilation system (non-recirculating) was used, mixing was inefficient, and uniform biological reduction within the cabin test section was not observed. With the known air circulation patterns of the AERF and other aircraft, this effect was expected. Based on this study, technology shows promise as an aircraft decontaminant.

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