Federal Aviation Administration

DOT/FAA/AM-09/7
Office of Aerospace Medicine
Washington, DC 20591

Inactivating Influenza Viruses on Surfaces Using Hydrogen Peroxide or Triethylene Glycol at Low Vapor Concentrations

Stephen N. Rudnick
James J. McDevitt
Melvin W. First
John D. Spengler

Air Transportation Center of Excellence for
Airliner Cabin Environment Research
Harvard School of Public Health
Boston, MA 02115

April 2009

Final Report

OK-09-0434-JAH

NOTICE

This publication and all Office of Aerospace Medicine technical reports are available in full-text from the Civil Aerospace Medical Institute's publications Web site:
www.faa.gov/library/reports/medical/oamtechreports

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-09/7		

4. Title and Subtitle	5. Report Date
Inactivating Influenza Viruses on Surfaces Using Hydrogen Peroxide or Triethylene Glycol at Low Vapor Concentrations	April 2009
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Rudnick SN, McDevitt JJ, First MW, Spengler JD	

9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
Air Transportation Center of Excellence for Airliner Cabin Environment Research Harvard School of Public Health 665 Huntington Avenue Boston, MA 02115	11. Contract or Grant No.

12. Sponsoring Agency name and Address	13. Type of Report and Period Covered
Office of Aerospace Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591	14. Sponsoring Agency Code

15. Supplemental Notes

Work was accomplished under Public law 108-76

16. Abstract

Any of the exposed surfaces in airplanes can become contaminated with infectious viruses, such as influenza, and facilitate transmission of disease. In this study, we disinfected surfaces contaminated with influenza viruses. Selection of the specific decontamination agents used in this study was based on three criteria: 1) no harm would be caused to the mechanical components or avionics of the airplane, 2) no potentially harmful residue would be left behind, and 3) the airplane could quickly be returned to service. We chose two decontamination agents that we believed fulfilled these criteria: 1) hydrogen peroxide (HP) at vapor concentrations in air below 100 ppm and 2) triethylene glycol (TEG) saturated air, which has a TEG vapor concentration of about 2 ppm at 25°C. For influenza viruses deposited on stainless-steel coupons and exposed for 15 min to 10 to 90 ppm of HP vapor, the number of log reductions of active viruses ranged from 3.6 to 4.7. The number of log reductions, however, was not linear with time; log reduction rate decreased significantly with increasing exposure time. For example, at a HP vapor concentration of 57 ppm, the number of log reductions was 3.2 after 2.5 min but just 4.0 after 10 min. Even after 60 min, the number of log reductions was only 5.6. At a HP vapor concentration of 10 ppm, the number of log reductions was 2.0 after 2.5 min. This corresponds to 99% inactivation of viruses, a significant reduction for such a low HP vapor concentration. For air saturated with TEG at 25-29°C, the number of log reductions of influenza viruses versus exposure time followed a linear relationship reasonably well. The decontamination rate was equal to 1.3 log reductions per hour. The potential for damage to the mechanical components or avionics of the airplane at a TEG vapor concentration of 2 ppm would be expected to be minimal. In addition, at a 2 ppm TEG vapor concentration, there is essentially no health risks to people.

17. Key Words	18. Distribution Statement
Surface Decontamination, Hydrogen Peroxide Vapor, Vaporized Hydrogen Peroxide, Triethylene Glycol Vapor, Influenza Virus	Document is available to the public through the Defense Technical Information Center, Ft. Belvoir, VA 22060; and the National Technical Information Service, Springfield, VA 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	14	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

ACKNOWLEDGMENTS

The authors thank Dr. William Gale for providing guidance during this project and for his assistance in procuring stainless-steel surfaces used during experiments. We also thank the technicians who worked on this project and contributed to its completion: Gihan Amarasiriwardena, Kenneth Chin, Alissa Fernald, Christa James, and Chika Uchiyama.

ABBREVIATIONS

As used in this report, the following abbreviations/acronyms have the meanings indicated

Abbreviation	Meaning
ACGIH	American Conference of Governmental Industrial Hygienists
BSA	Bovine serum albumin
DPBS	Dulbecco's phosphate buffered saline
DPBS++	DPBS with calcium and magnesium
EPA	Environmental Protection Agency
FFU	Fluorescent focus units
HP	Hydrogen peroxide
MDCK	Madin-Darby canine kidney
OSHA	Occupational Safety and Health Administration
PEL	Permissible exposure limit
RH	Relative humidity
SARS	Severe acute respiratory syndrome
TEG	Triethylene glycol
TLV®	Threshold Limit Value
UV	Ultraviolet
UVGI	Ultraviolet germicidal irradiation

MATHEMATICAL SYMBOLS

As used in this report, the following symbols have the meanings indicated

Symbol	Meaning
f	Fraction of viruses remaining active
$\overline{\log U}$	Mean of the logarithms of U
$\overline{\log U_o}$	Mean of the logarithms of U_o
n	Number of log reductions
$\bar{n}$	Average number of log reductions
p_{TEG}	Partial pressure of TEG in air
P	Ambient pressure
P^o_{TEG}	Vapor pressure of pure liquid TEG
$s_{\log U}$	Standard deviation of log U
$s_{\log U_o}$	Standard deviation of log U_o
s_n	Standard deviation corresponding to $\bar{n}$
T	Temperature
U	Number of FFU per volume of rinsate from an exposed coupon
U_o	Number of FFU per volume of rinsate from an unexposed coupon
y_{TEG}	TEG mole fraction in the gas phase

CONTENTS

Inactivating Influenza Viruses on Surfaces Using Hydrogen Peroxide or Triethylene Glycol at Low Vapor Concentrations

INTRODUCTION

Background

Preventing the spread of smallpox, influenza, SARS, and other virus-caused diseases on commercial airplanes and other public venues is a significant challenge for the public health community (Musher 2003; Olsen, Chang, Cheung, et al. 2003). Transfer of viruses from an infected individual to an uninfected one can occur through various modes of transmission: 1) by direct contact, 2) via fomites (inanimate objects capable of carrying infectious viruses), 3) from the direct spray of large droplets from an infected person, and 4) from droplet nuclei, which are very small dried droplets that can stay suspended in the air for long periods of time (Roy & Milton 2004). In this study, we evaluated the efficacy of various relatively gentle methods for decontaminating fomites.

Any of the exposed surfaces in airplanes or other vehicles used for public transportation can become contaminated with infectious viruses and be responsible for disease transmission. In this study, we disinfected surfaces contaminated with influenza A viruses, whose subtypes may have the potential to cause a pandemic propagated worldwide by commercial travel. It may not be necessary to sterilize an airplane cabin; significant reduction in the potential for disease transmission would be beneficial.

Selection of specific decontamination methods used in this study was based primarily on three criteria: 1) the method would not be expected to cause damage to the mechanical components or avionics of the airplane, 2) the method would leave no potentially harmful residue, and 3) the method would require a relatively brief period of time, so that an airplane or other means of public transportation could be put back into service quickly. We chose three methods that fulfill these criteria: 1) relatively low vapor concentrations (<100 ppm) of hydrogen peroxide (HP), 2) very low vapor concentrations of triethylene glycol (TEG), and 3) thermal decontamination using heated air. The efficacy of the decontaminants HP and TEG is the subject of this report. A separate report discusses thermal decontamination. As a baseline for comparison and because of its importance, the length of time that influenza viruses remain active on surfaces under ambient conditions was also determined.

We eliminated many other decontamination methods because they did not adhere to the desired criteria. Specifically, methods that rely on chlorine dioxide, formaldehyde, ethylene oxide, and methyl bromide were deemed likely unacceptable due to their potential for damage to the airplane and their toxicity to humans. In addition, despite its effectiveness for inactivating viruses and bacteria, we did not choose ultraviolet germicidal irradiation (UVGI) because viruses can be protected from UV rays by lurking in the shadows and because the UV rays are not very penetrating, allowing a coating of dust or other material to protect viruses from the UV rays. UVGI is more appropriate for air decontamination (First, Rudnick, Banahan, et al. 2007; Rudnick & First 2007).

Hydrogen Peroxide Vapor Decontamination

We were unable to find any peer-reviewed publications on surface decontamination of influenza viruses using HP at vapor concentrations below 100 ppm. In the few published studies on surface decontamination of influenza viruses at higher HP concentrations, the dried virus suspension was exposed to a relatively large dose of HP vapor; that is, the HP vapor concentration was relatively high and exposure time relatively long. None of these studies gave results on virus inactivation versus dose. For example, the effect of HP vapor on surface-deposited influenza viruses was evaluated by Heckert, Best, Jordan, et al. (1997) at a HP vapor concentration of about 1200 ppm and exposure time of about 30 min. Although Heckert, Best, Jordan, et al. showed an overall reduction of influenza viruses of about 6 logs, which was their limit of detection, only about 3 logs were attributable to HP vapor; the remainder was due to 16 h of drying at ambient conditions and heat exposure at 30-40°C. Other studies using influenza as the challenge viruses had similar limitations. In a recent review article, De Benedictis, Beato, and Capua (2007) conclude that "reports on the specific efficacy against avian influenza viruses of hydrogen peroxide are contradictory, and for this reason additional information on its viricidal efficacy is necessary."

Triethylene Glycol Vapor Decontamination

Although we were unable to find any publications on the use of TEG vapor to decontaminate surfaces, it has

been used to disinfect air. Although other glycols can also be used to disinfect air (Robertson, Bigg, Miller, et al. 1941; Robertson, Loosli, Puck, et al. 1941), TEG vapor is the most suitable because of its extremely low vapor pressure, which results in very low air concentrations. In addition, TEG in aerosol form is commonly used for theatrical "smoke" such as in venues for Broadway productions (Burr, Van Gilder, Trout, et al. 1994). As a result of this and other properties, TEG vapor is believed to do no harm to humans (EPA 2005a) or damage to environmental surfaces (Lester, Kaye, Robertson, et al. 1950). There are a large number of journal publications, primarily from the 1940s, on the use of TEG vapor for air disinfection. TEG vapor has been shown to exert lethal action against a wide variety of airborne infectious agents including bacteria, viruses, and fungi (Lester, Kaye, Robertson, et al. 1950). In particular, TEG vapor was found to be an effective decontaminant agent for airborne influenza viruses (Robertson, Puck, Lemon, et al. 1943).

METHOD AND MATERIALS

Influenza Virus

Virus Stock. A frozen suspension of influenza viruses (A/PR/8/34 H1N1) was purchased from Advanced Biotechnologies (Columbia, MD). It was thawed, divided into single-use packets, refrozen, and stored at −80°C until needed.

Virus Assay. A fluorescent focus reduction assay (Hartshorn, White, Tecle, et al. 2007) was used to measure the titer of virus suspensions before and after decontamination. Confluent monolayers of Madin-Darby canine kidney (MDCK) cells were prepared in 96-well plates. Each well was inoculated with 50 µL of coupon rinsate (or serial dilutions of the rinsate) and incubated at 37°C for 45 min in a 5% CO_2 environment. After washing the infected cells using assay media composed of Dulbecco's Modified Eagle's Medium (Mediatech, Herndon, VA) with 0.1% bovine serum albumin (BSA) (SeraCare, Milford, MA), the cells were incubated for 7 h at 37°C in a CO_2 environment. After incubation, the cells were washed three times with Dulbecco's phosphate buffer saline with calcium and magnesium (DPBS++) (Hyclone Laboratories, Logan, UT) and fixed with an aqueous solution of 80% acetone for 10 min at 4°C. The infected MDCK cells were then labeled for 30 min at 4°C with 50 µL of nucleoprotein antibody solution, which was made by adding 50 µL of mouse monoclonal antibodies (Centers for Disease Control, Atlanta, GA, catalog #VS2366) to 5 mL of Dulbecco's phosphate buffer saline (DPBS) (Hyclone Laboratories, Logan, UT) containing 1% BSA, 1% heat inactivated human serum (Mediatech, Herndon, VA), and 0.02% sodium azide. After washing three times with DPBS, the cells were incubated with tagging solution, which was made by adding 50 µL of rhodamine-labeled goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA, catalog #115026062) to 5 mL of DPBS containing 1% BSA, 1% heat inactivated human serum, and 0.02% sodium azide. The number of cells having a fluorescent foci, which are referred to as fluorescent focus units (FFU), were then counted using an Olympus CKX-41 inverted fluorescent microscope (Olympus, Center Valley, PA). Based on this assay, the titer of the single-use packets of influenza virus suspension after being thawed was about 10^9 FFU/mL.

Preparation and Treatment of Test Surfaces

One-inch by three-inch stainless-steel coupons were used as test surfaces. For tests using higher concentrations of HP vapor or TEG vapor, 50 µL of influenza virus suspension was seeded onto a predetermined number of coupons. For lower decontaminant concentrations, the influenza virus suspension was diluted prior to being seeded onto coupons. All of the coupons were placed into a biological safety cabinet until the deposited liquid had evaporated. The required drying time was between 20 and 30 min, depending on ambient conditions. Some of these seeded coupons, which are referred to as "control" coupons, were left in the biological safety cabinet, where they continued to be exposed to filtered room air at ambient conditions. The remaining seeded coupons (along with a clean coupon used as a negative control) were placed in an exposure chamber containing HP vapor or TEG vapor.

The coupons were removed from the exposure chamber after predetermined exposure times. The control coupons that were left in the biological safety cabinet had no exposure to HP or TEG and, thus, were considered to be unexposed coupons. Immediately after the last coupon was removed from the exposure chamber, each of the seeded coupons was rinsed with DPBS++ using the following procedure: The clearly marked portion of the coupon where the viruses had been initially deposited was rinsed 25 times with a single 500-µL portion of DPBS++ using a pipette. No visible residue remained. A fluorescent focus reduction assay was then done on the rinsate and/or diluted rinsate from each coupon. Only about 50% of the influenza viruses that were seeded onto the control slides were recovered.

Exposure Chamber and Test Methodology

Hydrogen Peroxide Vapor Tests. Tests in which influenza viruses deposited on stainless-steel coupons were exposed to HP vapor were done in a 130-L cubical plexiglass chamber located within a laboratory fume

hood. A shallow pool of an aqueous solution of 35% HP (VWR, West Chester, PA), diluted with water to a predetermined HP concentration that was calculated to provide the desired HP vapor concentration, covered much of the floor area of the chamber. Prediction of the HP concentration in an aqueous solution required for a specific HP vapor concentration was based on published correlations (Schumb, Satterfield & Wentworth 1955).

The air inside the chamber was kept well mixed through the use of two small fans. To maintain the desired relative humidity (RH), 17 L/min of dry air was added to the chamber. Temperature and RH were monitored and recorded every 30 s using a HOBO (Onset Computer Corp., Buzzards Bay, MA). RH and temperature were also measured periodically with a hygrometer (Omega Engineering, Stamford, CT) and mercury thermometer.

HP vapor concentration was monitored continuously and data logged using a newly purchased, calibrated ATI C16 PortaSenII with a hydrogen peroxide sensor (Analytical Technology, Collegeville, MD), which has a measurement range of 0 to 100 ppm HP vapor. The calibration was done on 9/6/07, directly before our experimental tests began; the manufacturer stated that the calibration had ±10% accuracy. Directly after our experimental tests were completed (1/30/08), the instrument was sent back to the manufacturer to be re-calibrated. The instrument was reading 17% higher than it should have been—again, within ±10% accuracy. Nevertheless, within the accuracy of the calibration method, the instrument's calibration remained stable during our experimental tests.

After a constant HP vapor concentration had been reached in the exposure chamber, test coupons were inserted into the chamber through a vertically opening sliding door. When inserting test coupons, the door was lifted only very slightly so that the HP vapor concentration would remain essentially constant.

Triethylene Glycol Vapor Tests. Tests in which influenza viruses deposited on stainless-steel coupons were exposed to TEG vapor were also done in the same well-mixed 130-L cubical plexiglass chamber used for the HP vapor tests. Greater care, however, was taken to seal the chamber, and dry air was not added to the chamber. A shallow pool of 99% pure liquid TEG (VWR, West Chester, PA) covered much of the floor area of the chamber. A beaker of water was placed within the chamber in order to help maintain a reasonably constant RH. If the beaker of water was not present, the RH in the chamber would decrease over time because TEG is very hydroscopic. Temperature and RH were monitored and recorded every 30 s using a HOBO (Onset Computer Corp., Buzzards Bay, MA). RH and temperature were also measured periodically with a hygrometer (Omega Engineering) and mercury thermometer. After equilibrium conditions had been achieved, test coupons were inserted into the chamber by minimally opening a vertically sliding door. The coupons were inserted as quickly as possible so as to minimize disruption of equilibrium conditions.

The concentration of TEG vapor was not measured. Because we allowed a large pool of nearly pure liquid TEG located on the floor of the well-mixed exposure chamber to reach equilibrium with the gas phase, the air was essentially saturated with TEG, and the partial pressure (p_{TEG}) of TEG was approximately equal to its vapor pressure (P^o_{TEG}). TEG vapor pressure can be calculated from the Antoine equation (NIST 2005):

$$\log_{10} P^o_{TEG} = 6.757 - \frac{3715}{T - 1.299} \qquad (1)$$

where vapor pressure is in bars and temperature (T) is in degrees Kelvin. Based on Equation 1, the vapor pressure of TEG at 25°C is 0.00131 mm Hg[1], which is in nearly perfect agreement with the value of 0.00132 mm Hg at 25°C given by the EPA (2005a). The TEG mole fraction (y_{TEG}) in the gas phase can be calculated from Dalton's law:

$$y_{TEG} = \frac{p_{TEG}}{P} \cong \frac{P^o_{TEG}}{P} = \frac{0.0013}{760} \times 10^6 = 1.7 \text{ ppm} \qquad (2)$$

where P is ambient pressure. In actuality, because liquid TEG is so hydroscopic, the pool of TEG on the chamber floor would tend to become diluted with water over time so that the mole fraction of TEG vapor would be somewhat less than 1.7 ppm. However, because the amount of liquid TEG in the chamber was relatively large, dilution would not be expected to have a very significant effect on the TEG vapor concentration.

Tests of Natural Die-Off Rate. Our normal methodology for evaluating the loss of virus activity over time involved seeding 50 µL of influenza virus suspension onto each of the coupons that were to be used during an experimental test. All virus assays for an experimental test were then done at the same time and, when possible, in the same 96-well plate. Because growing and maintaining cells is somewhat of an art, performing all assays for an experimental test at the same time is important in order to get consistent results.

For tests to measure the natural die-off rate of influenza viruses, this methodology could not be used because the duration of the test was too long, so an alternative procedure was employed. In preparation for an experimental test to measure the natural die-off rate of influenza viruses, single-use packets of influenza virus suspension

[1]Although Equation 1 was specified for a temperature range that did not include 25°C, it predicted the same vapor pressure at 25°C as was given by the EPA (2005a).

were thawed, separated into 200-µL portions, and then re-frozen at –80°C. At the start of a natural die-off test, one of these 200-µL portions was thawed, and then each of three stainless-steel coupons was seeded with 50 µL of virus suspension prior to being exposed to ambient conditions in a small chamber without a decontamination agent present. After a predetermined amount of time, another 200-µL portion was thawed, and three additional coupons were seeded and then exposed to ambient conditions. This procedure was repeated multiple times. At the end of the test, the virus residue on each coupon was extracted using our standard method, and each extraction was assayed at the same time and in the same plate.

Calculations

The number of fluorescent focus units (FFU) per volume of coupon rinsate is a measure of the quantity of cultureable viruses present on the coupon. The ratio of the number of FFU per volume in the rinsate from an exposed coupon (U) to the number from an unexposed coupon (U_o) is defined as the fraction of viruses remaining active (f):

$$f = \frac{U}{U_o} \tag{3}$$

For cultureable viruses, the number of log reductions (n) is equal to the difference between the logarithm of the initial FFU per volume and the logarithm of the final FFU per volume:

$$n = \log U_o - \log U = -\log f \tag{4}$$

where the logarithms are to the base 10. Thus, n = 4 corresponds to 4 log reductions, which is equivalent to 0.01% of the viruses remaining active and 99.99% of the viruses inactivated; that is, starting with 10,000 FFU in the rinsate from an unexposed coupon, only one would remain in the rinsate from the exposed coupon.

Because three coupons were exposed and three were not exposed during a specific time period, the unexposed and exposed coupons could not be separated into pairs. Therefore, the mean number of log reductions ($\overline{n}$) was calculated from Equation 5:

$$\overline{n} = \overline{\log U_o} - \overline{\log U} \tag{5}$$

where $\overline{\log U_o}$ and $\overline{\log U}$ are the means of the logarithms of U_o and U, respectively. The standard deviation (s_n) corresponding to $\overline{n}$ can be calculated from the standard deviation of log U_o ($s_{\log U_o}$) and the standard deviation of U ($s_{\log U}$):

$$s_n^2 = s_{\log U_o}^2 + s_{\log U}^2 \tag{6}$$

In figures in which the number of log reductions ($\overline{n}$) is plotted versus time, the error bars correspond to ±1 standard deviation (s_n), as given by Equation 6.

Based on 10^9 FFU/mL for the influenza virus suspension in a single-use packet and 50% recovery of viruses from control slides, the theoretical limit of detection in terms of the number of log reductions that could be detected by the methods described above was calculated to be 7.4. This calculation is based on the assumption that a single FFU detected from any of the three coupons exposed at a specific test condition corresponds to the limit of detection.

RESULTS

Hydrogen Peroxide Vapor Decontamination

The number of log reductions based on Equation 5 versus exposure time for tests in which influenza viruses deposited on stainless-steel coupons were exposed at approximately 25°C and 58-65% RH to relatively low concentrations of HP vapor is shown in Figure 1. In this figure, error bars correspond to ± one standard deviation, based on Equation 6. Even at a HP vapor concentration as low as 10 ppm, about a two-log reduction was observed after 2.5 min of exposure. The reduction, however, did not increase as much as would be expected with increases in either exposure time or HP vapor concentration. If a HP vapor concentration of 10 ppm and an exposure time of 2.5 min are taken as the base, increasing exposure time by a factor of six or concentration by a factor of nine added only an extra 1.6 and 1.3 logs of reduction, respectively. For 15 min of exposure time, the highest measured decontamination rate was 4.7 log reductions at a HP vapor concentration of 90 ppm. An additional test, not shown in Figure 1, in which influenza viruses were exposed at a HP vapor concentration of 57 ppm for 60 min, resulted in a decontamination rate of 5.6 log reductions.

Triethylene Glycol Vapor Decontamination

The number of log reductions based on Equation 5 as a function of exposure time for tests in which influenza viruses deposited on stainless-steel coupons were exposed to air saturated with TEG at 25-29°C and 45-55% RH is shown in Figure 2. Based on Equations 1 and 2, the concentration of TEG vapor in these tests was equal to between 1.7 and 2.5 ppm. Error bars in Figure 2 correspond to ± one standard deviation, based on Equation 6. The number of log reductions (n) versus exposure time (t) follows a linear relationship reasonably well. The relationship is given by the following equation:

$$n = 1.31t \tag{7}$$

where exposure time is in hours. Thus, the decontamination rate attributable to TEG vapor was 1.3 log reductions

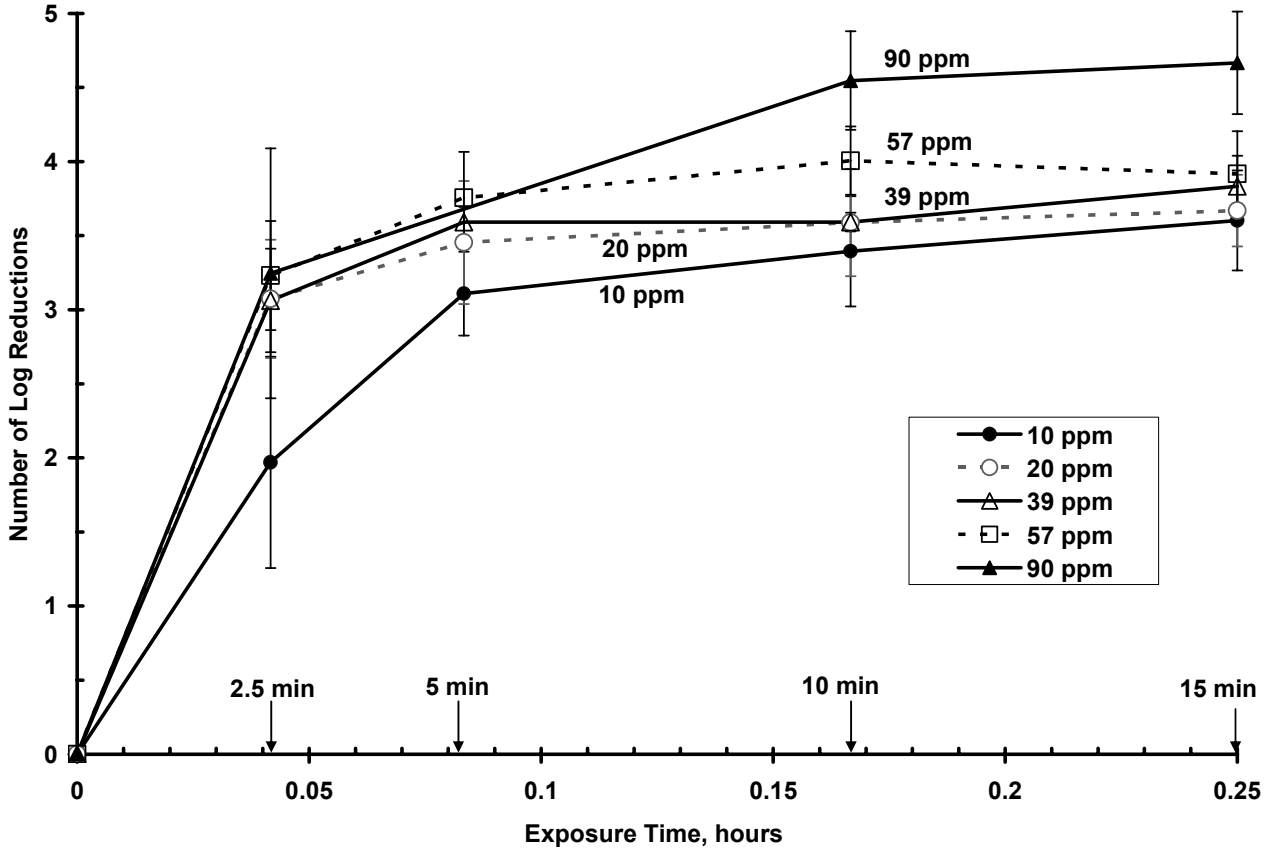

Figure 1. Surface decontamination of influenza viruses with hydrogen peroxide vapor

per hour. Equation 7 is equivalent to Equation 8, the equation for exponential decay of the fraction of viruses remaining active (f) :

$$f = \exp(-3.02t) \quad (8)$$

Viability Tests at Ambient Conditions

For purposes of comparison with chemical decontamination tests, the natural die-off rate at ambient conditions of influenza viruses deposited on stainless-steel coupons was measured. The number of log reductions versus time for two separate tests, each lasting a few days, is plotted in Figure 3.

Error bars in this figure correspond to ± one standard deviation based on Equation 6. No decontamination agent was used during these tests. Based on the data points from both tests, the number of log reductions (n) versus exposure time (t) follows a linear relationship given by Equation 9:

$$n = 0.0829\,t \quad (9)$$

where exposure time is in hours. Thus, the natural decay rate of influenza viruses was 0.083 log reductions per hour, which is equivalent to a half-life of 3.6 h.

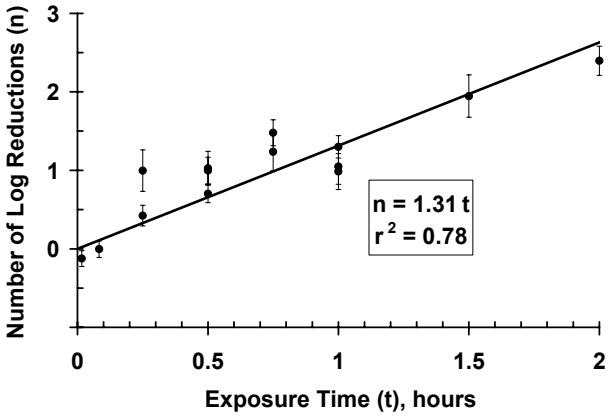

Figure 2. Surface decontamination of influenza viruses with triethylene glycol saturated air

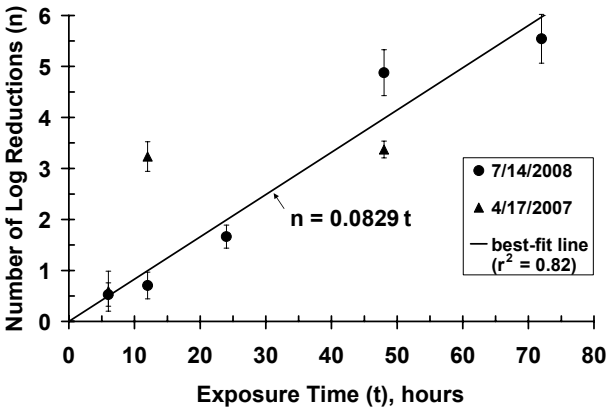

Figure 3. Natural die-off of influenza viruses on stainless-steel coupons at ambient conditions

DISCUSSION

Hydrogen Peroxide Vapor Decontamination

Test results on the decontamination of influenza viruses using HP vapor at concentrations less than 100 ppm (Fig. 1) are somewhat surprising in that the number of log reductions in active viruses versus exposure time is very non-linear; that is, the fraction of viruses remaining active versus exposure time does not follow an exponential decay curve. As the exposure time increases, the log reduction rate decreases significantly; thus, as shown in Figure 1, the number of log reductions for the initial 2.5 min of exposure is greater than the number of log reductions from 2.5 to 15 min of exposure. This trend is true for all HP vapor concentrations evaluated. For example, at a HP vapor concentration of 10 ppm, the lowest concentration tested, the number of log reductions was 2.0, 3.1, 3.4, and 3.6 after 2.5, 5, 10, and 15 min of exposure, respectively. Similarly, at a HP vapor concentration of 90 ppm, the highest concentration tested, the number of log reductions was 3.2, 4.5, and 4.7 after 2.5, 10, and 15 min of exposure, respectively.

Another unexpected outcome of these tests was that, in the initial 2.5 min of exposure to 10 ppm HP vapor, the number of log reductions was equal to two, which is a 99% virus reduction. If the number of log reductions at 10 ppm HP vapor versus exposure time was linear, 15 min of exposure would result in sterilization (12 log reductions). Instead, due to the nonlinearity of the curves, after 15 min of exposure to 10 ppm HP vapor, only 3.6 log reductions were measured. Nevertheless, this is a significant reduction for such a low HP vapor concentration. The Threshold Limit Value (TLV®) and OSHA-permissible exposure limit (PEL) for occupational HP vapor exposure is an eight-hour time-weighted average of 1 ppm (ACGIH 2008; OSHA 2008). This suggests that 10 ppm of HP vapor is a relatively safe concentration over a short time period, although the TLV includes the caveat that HP vapor is a "confirmed animal carcinogen with unknown relevance to humans."

Triethylene Glycol Vapor Decontamination

Dividing Equation 7 by Equation 9 indicates that TEG vapor increases the natural die-off rate of influenza viruses by a factor of 16. The decontamination rate for air saturated with TEG vapor at 25-29°C, which was measured to be 1.3 log reductions per hour, is considerably less than for HP vapor (Fig. 1), even at a concentration of 10 ppm. For example, for a 15-minute exposure period, the decontamination rate for TEG vapor was 0.33 log reductions, compared to 3.6 log reductions for HP vapor. Nevertheless, TEG vapor has some important advantages.

For surface decontamination using TEG vapor, ambient air or warmed air could easily be saturated with TEG prior to being introduced into an airplane cabin. Alternatively, micrometer-size TEG droplets, which evaporate rapidly, could be injected into the supply air duct or directly into the cabin air. The standard method for introducing TEG droplets into air for the purpose of air decontamination is through the use of a pressurized liquid (EPA 2005a), although a nebulizer could also be used. If a pandemic were to occur, both surface and air decontamination could take place simultaneously, even while passengers were onboard.

Although an objection could be raised due to the potential health risk of using TEG vapor for air decontamination, this is likely an unwarranted concern because TEG is an odorless chemical of no known toxicity, and exposure of people to TEG is already widespread. TEG vapor is used as a bacteriostat to kill odor-causing bacteria for the purpose of air sanitation and deodorization. It was first registered for use in hospitals as an air disinfectant in 1947. Present application scenarios include spraying TEG inside offices, schools, hotels, lobbies, theaters, reception rooms, sleeping rooms, bathrooms, and hospital rooms (EPA 2005b). In addition, products containing TEG packaged in aerosol cans and designed to be sprayed into the air inside homes to control odors are sold in stores everywhere (e.g., Oust® and Febreze®).

According to the U.S. Environmental Protection Agency (EPA 2005a), "the Agency has no risk concerns for triethylene glycol with respect to human exposure. Based on a review of the available toxicology data, the Agency has concluded that triethylene glycol is of very low toxicity by the oral, dermal, and inhalation routes

of exposure. The toxicology database is adequate to characterize the hazard of triethylene glycol, and no data gaps have been identified. There are no indications of special sensitivity of infants or children resulting from exposure to triethylene glycol." In addition, TEG has no known deleterious effects on fabrics or other surfaces (Lester, Kaye, Robertson & Dunklin 1950). Unlike HP vapor, TEG vapor is not an oxidizing agent. TEG inactivates viruses and bacteria because it is very hydroscopic; it condenses on bacteria- and virus-containing particles until the TEG concentration becomes sufficiently high to be germicidal (Puck, T.T. 1947a; Puck, T.T. 1947b).

To demonstrate the effectiveness of simultaneous surface and air decontamination, microbiological studies need to be conducted in a room-size chamber using both viruses and bacteria as challenges. Methods also need to be developed to monitor TEG vapor concentration so that TEG introduction can be precisely controlled.

It is reasonable to expect that the efficacy of TEG vapor will increase as its concentration is increased. However, at 25°C, the concentration of TEG in air cannot exceed 1.7 ppm because air is saturated at that concentration. The only way of increasing the concentration is to increase temperature. As shown in Table 1, which was calculated from Equations 1 and 2, modest increases in temperature result in significant increases in TEG vapor concentration. Thus, further work investigating TEG vapor as a decontaminating agent is warranted. Specifically, the effectiveness of TEG vapor for surface decontamination at higher concentrations—that is, at temperatures greater than room temperature—should be determined. In addition, the influence of RH on decontamination effectiveness should also be evaluated.

Table 1. Triethylene Glycol Concentration in Air

Temperature, °C	Concentration, ppm
20	1.1
25	1.7
30	2.8
35	4.4
40	6.9

CONCLUSIONS

Our experiments show that HP vapor concentrations as low as 10 ppm and TEG vapor concentrations of 2 ppm can provide effective decontamination of a commercial airplane cabin. At these very low concentrations, the potential for damage to the mechanical components or avionics of the airplane would be expected to be minimal. Although it has somewhat lower efficacy than 10 ppm HP vapor, air saturated with TEG vapor at 25°C is probably the better choice for decontamination of airplane cabins; TEG is safer with regards to both personnel and airplanes and is easier to apply. If TEG vapor is determined to be a viable candidate for decontamination of airplane cabins, optimizing temperature and RH would likely lead to greater efficacy.

REFERENCES

ACGIH (2008). *TLVs and BEIs*, American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

Burr, G.A., Van Gilder, T.J., Trout D.B., Wilcox, T.G., and Driscoll, R.M. (1994). Health hazard evaluation, HETA 90-0355-2449. National Institute of Occupational Health and Safety.

De Benedictis, P., Beato, M.S., and Capua, I. (2007). Inactivation of avian influenza viruses by chemical agents and physical conditions: a review. *Zoonoses Public Health*, 54(2), 51-68.

EPA (2005a). *Reregistration eligibility decision for triethylene glycol*, EPA739-R-05-002, United States Environmental Protection Agency.

EPA (2005b). *Revised antimicrobials division's review of the disciplinary sciences for issuance of the reregistration eligibility decision* (RED) document, EPA-HQ-OPP-2005-0002, United States Environmental Protection Agency.

First, M.W., Rudnick, S.N., Banahan, K.F., Vincent, R.L., and Brickner, P.W. (2007). Fundamental factors affecting upper room ultraviolet germicidal irradiation—Part I. Experimental, *J Occup Environ Hyg,* 4(5), 321-31.

Hartshorn, K.L., White, M.R., Tecle, T., Tornoe, I., Sorensen, G.L., Crouch, E.C., and Holmskov, U. (2007). Reduced influenza viral neutralizing activity of natural human trimers of surfactant protein D. *Respir Res*, 8(1), 9-19.

Heckert, R.A., Best, M., Jordan, L.T., Dulac, G.C., Eddington, D.L., and Sterritt, W.G. (1997). Efficacy of vaporized hydrogen peroxide against exotic animal viruses. *Appl Environ Microbiol*, 63(10), 3916-8.

Lester, W., Kaye, S, Robertson, O.H., and Dunklin, E.W. (1950). Factors of importance in the use of triethylene glycol vapor for aerial disinfection. *Am J Pub Health*, 40(7), 813-20.

Musher, D.M. (2003). How contagious are common respiratory tract infections? *N Engl J Med*, 348, 1256-66.

NIST (2005). Chemistry WebBook, National Institute of Standards and Technology. <webbook.nist.gov/cgi/cbook.cgi?ID=C112276&Units=SI&Mask=4#Thermo-Phase>.

Olsen, S.J., Chang, H.L., Cheung, T.Y.Y., Tang, A.F.Y., Fisk, T.L., Ooi, S.P.L., Kuo, H.W., Jiang, D.D.S., Chen, K.T., Lando, J., Hsu, K.H.H., Chen, T.J., and Dowell, S.F. (2003). Transmission of the severe acute respiratory syndrome on aircraft. *N Engl J Med,* 349, 2416-22.

OSHA (2008). Limits for air contaminants, Standard Number 29 CFR 1910.1000, Table Z-1, Electronic Code of Federal Regulations. <http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c= ecfr&rgn=div5&view=text&node=29:6.1.1.1.1&idno=29#29:6.1.1.1.1.1.1.1>.

Puck, T.T. (1947a). The mechanism of aerial disinfection by glycols and other chemical agents I. Demonstration that the germicidal action occurs through the agency of the vapor phase. *J Exp Med*, 85, 729-39.

Puck, T.T. (1947b). The mechanism of aerial disinfection by glycols and other chemical agents II. An analysis of the factors governing the efficiency of chemical disinfection of the air. *J Exp Med,* 85, 741-57.

Robertson, O.H., Loosli, C.G., Puck, T.T., Bigg, E., and Miller, B.F. (1941). The protection of mice against infection with air-borne influenza virus by means of propylene glycol vapor. *Science,* 94 (2452), 612-3.

Robertson, O.H., Bigg, E., Miller, B.F., Baker, Z., and Puck, T.T. (1941). Sterilization of air by certain glycols employed as aerosols. *Science,* 93(2409), 213-4.

Robertson, O.H., Puck, T.T., Lemon, H.F., and Loosli, C.G. (1943). The lethal effect of triethylene glycol on air-borne bacteria and influenza virus. *Science,* 97(2510), 142.-4.

Roy, C.J. and Milton, D.K. (2004). Airborne transmission of communicable infection—the elusive pathway. *N Engl J Med*, 350, 1710-2.

Rudnick, S.N. and First, M.W. (2007). Fundamental factors affecting upper-room ultraviolet germicidal irradiation—Part II. predicting effectiveness. *J Occup Environ Hyg*, 4(5), 352-62.

Schumb, W.C., Satterfield, C.N., and Wentworth, R.L. (1955). *Hydrogen peroxide*, American Chemical Society Monograph, series no. 128, Reinhold Publishing Corp., New York.