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Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations

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Background: Surfaces in congregate settings, such as vehicles used for mass transportation, can become contaminated with infectious microorganisms and facilitate disease transmission. We disinfected surfaces contaminated with H1N1 influenza viruses using hydrogen peroxide (HP) vapor at concentrations below 100 ppm and triethylene glycol (TEG)-saturated air containing 2 ppm of TEG at 25°C. *Methods*Influenza viruses in aqueous suspensions were deposited on stainless-steel coupons, allowed to dry at ambient conditions, and then exposed for up to 15 minutes to 10 to 90 ppm of HP vapor or TEG-saturated air. Virus assays were done on the solution used to wash the viruses from these coupons and from coupons treated similarly but without exposure to HP or TEG vapor.

Results: After 2.5 minutes, exposure to 10-ppm HP vapor resulted in 99% inactivation. For air saturated with TEG at 25 to 29° C, the disinfection rate was about 1.3 log₁₀ reductions per hour, about 16 times faster than the measured natural inactivation rate under ambient conditions.

Conclusions: Vapor concentrations of 10 ppm HP or 2 ppm TEG can provide effective surface disinfection. At these low concentrations, the potential for damage to even the avionics of an airplane would be expected to be minimal. At a TEG vapor concentration of 2 ppm, there are essentially no health risks to people.

Key Words: Surface disinfection; fomites; influenza virus; hydrogen peroxide vapor; triethylene glycol.

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Preventing the spread of smallpox, influenza, severe acute respiratory syndrome, and other virus-caused diseases on commercial airplanes and in other public venues is a significant challenge for the public health community.^{1,2} Transfer of viruses from an infected individual to an uninfected individual can occur through various modes of transmission, including direct contact, fomites (inanimate objects capable of carrying infectious viruses), direct spray of large droplets from an infected person, and droplet nuclei, very small dried droplets that can stay suspended in air for long periods of time.^{3,4} In the present study, we evaluated the efficacy of various relatively gentle methods for disinfection of fomites.

Any of the exposed surfaces in vehicles used for mass transportation and other indoor congregate spaces can become contaminated with infectious viruses and be

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responsible for disease transmission. For example, influenza viruses have been shown to have the capability to survive on banknotes for very long periods.⁵ They also can remain active with minimal reduction after 60 minutes on human hands, although washing with soap and water is a highly effective hygiene strategy.⁶ In the present study, we disinfected surfaces contaminated with H1N1 influenza A viruses, whose subtypes may have the potential to cause a pandemic propagated worldwide by commercial travel. Sterilizing surfaces is not necessary; a significant reduction in the potential for disease transmission will be very beneficial.

Selection of specific disinfection methods used in this study was based primarily on 3 criteria: The method would be expected to (a) not cause damage to airplane mechanical components or avionics, (b) leave no potentially harmful residue, and (c) take a relatively brief period of time, so that an airplane or other means of public transportation could be returned to service quickly. We chose 2 methods that fulfilled these criteria: relatively low vapor concentrations (< 100 ppm) of hydrogen peroxide (HP) and very low vapor concentrations of triethylene glycol (TEG). The efficacy of HP and TEG is the subject of this study. In addition, as baseline for comparison and because of its importance, the length of time that influenza viruses remain active on surfaces under ambient indoor conditions was determined.

We were unable to find any peer-reviewed publications on surface disinfection of influenza viruses using HP at vapor concentrations < 100 ppm. In the few published studies on surface disinfection of influenza viruses at higher HP concentrations, dried virus suspension was exposed to a relatively large dose of HP vapor; that is, HP vapor concentration was relatively high and exposure time was relatively long. None of these studies provided results on virus inactivation versus dose. For example, the effect of HP vapor on surface-deposited influenza viruses was evaluated by Heckert et al⁷ at a HP vapor concentration of about 1200 ppm and an exposure time of about 30 minutes. Although they reported an overall reduction of influenza viruses of about 6 logs (base 10), which was their limit of detection, only about 3 logs were attributable to HP vapor; the remainder was due to 16 hours of drying at ambient conditions and heat exposure at 30 to 40°C. Other studies using influenza as the challenge virus had similar limitations. In a recent review article, De Benedictis et al⁸ concluded that reports on the specific efficacy against avian influenza viruses of hydrogen peroxide are contradictory; thus, additional information on its viricidal efficacy is needed.

Although we were unable to find any publications on the use of TEG vapor to disinfect surfaces, TEG vapor has been used to disinfect air. Although other glycols also can be used to disinfect air,^{9,10} TEG vapor is the most suitable because of its extremely low vapor pressure, which makes it effective at very low air concentrations. In addition, TEG in aerosol form is commonly used for theatrical "smoke," such as in venues for Broadway productions.¹¹ Because of these and other properties, TEG vapor is believed to cause no harm to humans¹² and no damage to environmental surfaces.¹³ There are numerous journal reports, 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.¹³ In particular, TEG vapor is an effective disinfectant agent for airborne influenza viruses.¹⁴

METHODS

Influenza virus

A frozen suspension of influenza viruses (A/PR/8/34 H1N1; purchased from Advanced Biotechnologies, Columbia, MD) was thawed, divided into single-use packets, refrozen, and stored at -80°C until needed. A fluorescent focus reduction assay¹⁵ was used to measure virus titer before and after disinfection. For this assay, confluent monolayers of Madin-Darby canine kidney (MDCK) cells were prepared in 96-well plates. Each well was inoculated with 50 μ L of a virus

suspension and incubated at 37°C for 45 minutes in a 5% CO₂ environment. After infected cells were washed with an assay medium comprising 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 hours at 37°C in a 5% CO₂ environment. After incubation, the cells were washed 3 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 minutes at 4°C. Infected MDCK cells were then labeled for 30 minutes at 4°C with 50 µL of nucleoprotein antibody solution, which was made by adding 50 µL of mouse monoclonal antibodies (catalog no. VS2366; Centers for Disease Control, Atlanta, GA) to 5 mL of Dulbecco's phosphatebuffered saline (DPBS) (Hyclone Laboratories) containing 1% BSA, 1% heat-inactivated human serum (Mediatech), and 0.02% sodium azide. After 3 washes with DPBS, the cells were incubated with tagging solution, made by adding 50 µL of rhodamine-labeled goat anti-mouse IgG (catalog no. 115026062; Jackson ImmunoResearch Laboratories, West Grove, PA) to 5 mL of DPBS containing 1% BSA, 1% heat-inactivated human serum, and 0.02% sodium azide. The number of cells with fluorescent foci, referred to as fluorescent focus units (FFUs), was 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 thawing was about 10⁹ FFUs/mL.

Preparation and treatment of test surfaces

Stainless steel coupons $1" \times 3"$ in size, typical of the stainless steel used in newer commercial airline cabins, were used as the test surfaces. For experiments using higher concentrations of HP vapor or TEG vapor, 50 µL of influenza virus suspension was seeded onto a predetermined number of coupons. For the experiments using lower disinfectant concentrations, the influenza virus suspension was diluted before being seeded onto the coupons. All coupons were kept in a biological safety cabinet until the deposited liquid had evaporated. The required drying time was 20 to 30 minutes, depending on ambient conditions. Some of these seeded coupons, referred to as "control" coupons, were kept in the biological safety cabinet and continually 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.

Groups of 3 coupons were removed from the exposure chamber after predetermined exposure times. The control coupons that were kept in the biological safety cabinet were not exposed to HP or TEG and thus were considered unexposed coupons. Immediately after the last 3 coupons were removed from the exposure chamber, each seeded coupon was washed with DPBS++ using the following procedure. The clearly marked portion of the coupon where viruses had been initially deposited was washed 25 times with a single 500- μ L portion of DPBS++ using a pipette. No visible residue remained. Virus assays were then done on the DPBS++ and/or the diluted DPBS++ used to wash each coupon. Only about 50% of the influenza viruses that were seeded onto the control slides were recovered.

HP vapor tests

Experiments in which influenza viruses deposited on stainless steel coupons were exposed to HP vapor were performed 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 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.¹⁶

The air inside the chamber was kept well mixed through the use of 2 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 seconds using a HOBO temperature/ RH data logger (model H08-003-02; Onset Computer Corp, Buzzards Bay, MA). RH and temperature also were measured periodically with a hygrometer (Omega Engineering, Stamford, CT) and a mercury thermometer.

HP vapor concentration was monitored continuously, and data were logged using a newly purchased, calibrated ATI C16 PortaSenII with an HP sensor (Analytical Technology, Collegeville, PA), which has an HP vapor measurement range of 0 to 100 ppm. The instrument was calibrated directly before the start of the experiments; according to the manufacturer, the calibration had an accuracy of $\pm 10\%$. About 5 months later, immediately after the completion of our experiments, the instrument was sent back to the manufacturer for recalibration. The instrument read 17% higher than it should have, but within $\pm 10\%$ accuracy. Thus, within the accuracy of the calibration method, the instrument's calibration remained stable during our experiments.

After a constant HP vapor concentration was reached in the exposure chamber, test coupons were inserted into the chamber through a vertically opening sliding door. While inserting test coupons, the door was lifted only very slightly, keeping the HP vapor concentration essentially constant.

TEG vapor tests

Experiments in which influenza viruses deposited on stainless steel coupons were exposed to TEG vapor were done in the same well-mixed chamber used for the HP vapor experiments. Greater care was taken to seal the chamber, however, and dry air was not added to the chamber. A shallow pool of 99% pure liquid TEG (VWR) covered much of the chamber's floor area. A beaker of water was placed within the chamber to help maintain a reasonably constant RH. Without the beaker of water, the RH in the chamber would decrease over time, because TEG is very hydroscopic. The chamber was left overnight to ensure equilibrium conditions. Test coupons were then inserted into the chamber by opening the sliding door only slightly, 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 of TEG (p_{TEG}) was approximately equal to its vapor pressure (P_{TEG}^o). TEG vapor pressure can be calculated from the Antoine equation,¹⁷

$$\log_{10} P^o_{TEG} = 6.757 - \frac{3715}{T - 1.299},\tag{1}$$

where vapor pressure is in bars and absolute temperature (*T*) is in°K. Based on eq (1), which was specified for a temperature range that did not include 25°C, the vapor pressure of TEG at 25°C is 0.00131 mm Hg, which is in nearly perfect agreement with the value of 0.00132 mm Hg at 25°C given by the Environmental Protection Agency (EPA).¹² The TEG mole fraction (y_{TEG}) in the gas phase can be calculated from Dalton's law,

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

where *P* is ambient pressure. Actually, 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. But because of the relatively large amount of liquid TEG in the chamber, dilution would not be expected to have a very significant effect on TEG vapor concentration.

Tests of natural inactivation rate

Our usual methodology for evaluating the loss of virus activity over time involved seeding 50 μ L of influenza virus suspension at essentially the same time

onto each coupon to be used during an experiment. All virus assays for an experiment were then performed at the same time, usually in the same 96-well plate. Because growing and maintaining cells is somewhat of an art, performing all assays for an experiment at the same time is important to getting consistent results.

Because of the extended duration of the experiment, we could not use this methodology to measure the natural inactivation rate of influenza viruses; thus, we used an alternative procedure. In preparation for an experiment to measure the natural inactivation rate of influenza viruses, single-use packets of influenza virus suspension were thawed, separated into 200-µL portions, and then refrozen at -80°C. At the start of a natural inactivation experiment, one of these 200-µL portions was thawed, and then each of 3 stainless steel coupons was seeded with 50 µL of virus suspension before being exposed to ambient indoor conditions in a small chamber without a disinfection agent present. After a predetermined time, another 200-µL portion was thawed, and 3 additional coupons were seeded and then placed in the chamber. This procedure was repeated multiple times. At the end of the experiment, the virus residue on each coupon was extracted, and all residues were assayed at the same time and in the same plate.

Calculations

The number of FFUs per volume of DPBS++ used to wash a coupon is a measure of the quantity of cultureable viruses present on the unwashed coupon. The ratio of the FFUs per volume of DPBS++ used to wash an exposed coupon (U) to that used to wash an unexposed coupon (U_o) is defined as the fraction of viruses remaining active (f),

$$f = \frac{U}{U_o}.$$
(3)

The number of \log_{10} reductions (*n*) is equal to the difference between the logarithm of the FFU per volume for unexposed and exposed coupons,

$$n = \log_{10} U_o - \log_{10} U = -\log_{10} f, \tag{4}$$

where logarithms are always to base 10. Thus, n = 4 corresponds to 4 log₁₀ reductions, which is equivalent to 0.01% of viruses remaining active and 99.99% of viruses being inactivated; that is, starting with 10,000 FFUs in the DPBS++ used to wash an unexposed coupon, only 1 FFU would remain in the DPBS++ used to wash the exposed coupon.

Because 3 coupons were exposed and 3 coupons were not exposed during any specific time period, unexposed and exposed coupons could not be separated into pairs. Therefore, the mean number of \log_{10} reductions (\overline{n}) was calculated from

$$\overline{n} = \overline{\log_{10} U_o} - \overline{\log_{10} U},\tag{5}$$

where $\overline{\log_{10} U_o}$ and $\overline{\log_{10} U}$ are the means of logarithms of U_o and U, respectively. The standard deviation (SD), s_n , corresponding to \overline{n} can be calculated from the SDs of $\log_{10} U_o$ ($s_{\log U_o}$) and $\log_{10} U$ ($s_{\log U}$),

$$s_n = \sqrt{s_{\log U}^2 + s_{\log U}^2}.$$
 (6)

Thus, s_n is a measure of the experimental variability for identical coupons used during a single experiment conducted under a specific set of operating conditions. It does not include experimental variability arising from replication of the experiment at a different time under the same set of operating conditions.

Based on 10^9 FFUs/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_{10} reductions that could be detected by the aforementioned method was calculated to be 7.4. This calculation is based on the assumption that a single FFU detected from any of the 3 coupons exposed at a specific test condition corresponds to the limit of detection.

RESULTS

HP vapor disinfection

Figure 1 shows the number of log₁₀ reductions based on eq (5) versus exposure time for experiments in which influenza viruses deposited on stainless steel coupons were exposed at approximately 25°C and 58% to 65% RH to relatively low concentrations of HP vapor. In this figure, error bars correspond to \pm 1 SD based on eq (6). Even at a HP vapor concentration as low as 10 ppm, about a 2-log₁₀ reduction was observed after 2.5 minutes of exposure. But the number of log₁₀ reductions did not increase with increases in either exposure time or HP vapor concentration as much as would be predicted based on a linear relationship. If a HP vapor concentration of 10 ppm and an exposure time of 2.5 minutes were taken as the base, then increasing exposure time by a factor of 6 or increasing concentration by a factor of 9 added only an extra 1.6 log₁₀ and 1.3 log₁₀ reduction, respectively. For 15 minutes of exposure time, the highest measured disinfection rate was a 4.7 \log_{10} reduction at a HP vapor concentration of 90 ppm. An additional experiment, not shown in Figure 1, in which influenza viruses were exposed at a HP vapor concentration of 57 ppm for 60 minutes resulted in a 5.6 \log_{10} reduction.



Fig I. Surface disinfection of influenza viruses with HP vapor. Error bars correspond to ± 1 SD based on tests using multiple coupons.

TEG vapor disinfection

Figure 2 shows the number of \log_{10} reductions based on eq (5) as a function of exposure time for experiments in which influenza viruses deposited on stainless steel coupons were exposed to air saturated with TEG at 25 to 29°C and 45% to 55% RH. Based on eqs (1) and (2), the concentration of TEG vapor in these experiments ranged from 1.7 to 2.5 ppm. The error bars in Figure 2 correspond to \pm 1 SD based on eq (6). The number of \log_{10} reductions (*n*) versus exposure time (*t*) appears to follow a linear relationship given by

$$n = 1.31 t,$$
 (7)

with exposure time in hours. This linear regression line has been forced through the origin; however, there is sufficient scatter around this regression line to make a nonlinear relationship a distinct possibility. Nevertheless, the disinfection rate attributable to TEG vapor can be approximated as $1.31 \log_{10}$ reductions per hour (with a 95% confidence interval [CI] of 1.11 to 1.52). Equation (7) is equivalent to eq (8), the equation for exponential decay of the fraction of viruses remaining active (*f*),

$$f = \exp\left(-3.02t\right). \tag{8}$$

Inactivation tests under ambient conditions

For the purpose of comparison with chemical disinfection experiments, we measured the natural inactivation rate at ambient indoor conditions of influenza viruses deposited on stainless steel coupons. Figure 3 plots the number of \log_{10} reductions versus time for 2 separate experiments done 9 months apart. Although only 2 of the data points in the figure are associated with the earlier experiment, other coupons exposed in this experiment for 96 hours or longer were found to be virus-free. At 96 hours, a \log_{10} reduction of > 5.6 (the limit of detection for the test) is not inconsistent with the regression line shown in the figure. The error bars in the figure correspond to \pm 1 SD based on eq (6). Based on data points from both experiments, the number of \log_{10} reductions (*n*) versus exposure time (*t*) can be approximated with a linear relationship given by

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

where exposure time is in hours. This linear regression line has been forced through the origin; however, the scatter around this regression line suggests that a nonlinear relationship is plausible as well. Nonetheless, the natural decay rate of influenza viruses can be approximated as 0.083 \log_{10} reductions per hour (95% CI = 0.061 to 0.105), which is equivalent to a half-life of 3.6 hours.

DISCUSSION

HP vapor disinfection

The experimental results for the disinfection of surfaces contaminated with influenza viruses using HP vapor at concentrations < 100 ppm, shown in Figure 1, indicate that the number of \log_{10} reductions of active viruses versus exposure time is very nonlinear; that is, the fraction of viruses remaining active versus



Fig 2. Surface disinfection of influenza viruses with TEG-saturated air. Error bars correspond to \pm 1 SD based on tests using multiple coupons.

exposure time does not follow an exponential decay curve. As exposure time increases, the \log_{10} reduction rate decreases significantly; thus, as shown in Figure 1, the number of \log_{10} reductions for the initial 2.5 minutes of exposure exceeds the number of \log_{10} reductions from 2.5 to 15 minutes of exposure. This trend holds for all HP vapor concentrations evaluated. For example, at a HP vapor concentration of 90 ppm (the highest concentration tested), the number of \log_{10} reductions was 3.2 after 2.5 minutes of exposure, 4.5 after 10 minutes of exposure, and 4.7 after 15 minutes of exposure.

Nonetheless, an important outcome of these tests was that during the initial 2.5 minutes of exposure to 10-ppm HP vapor, the number of \log_{10} reductions was equal to 2, or equivalently, a 99% reduction in virus. If the number of log₁₀ reductions at 10 ppm HP vapor versus exposure time were linear, then 15 minutes of exposure would have resulted in sterilization (12 \log_{10} reductions). Instead, because of the nonlinearity of the curve, only 3.6 log₁₀ reductions were measured after 15 minutes of exposure to 10-ppm HP vapor. This is a significant reduction for such a low HP vapor concentration. The 8-hour time-weighted average threshold limit value (TLV)¹⁸ and Occupational Safety and Health Administration permissible exposure limit¹⁹ for occupational HP vapor exposure are both 1 ppm. This suggests that 10-ppm HP vapor is a relatively safe concentration over a brief period, although the TLV includes the caveat that HP vapor is a confirmed animal carcinogen with unknown relevance to humans.

TG vapor disinfection

Dividing eq (7) by eq (9) indicates that TEG vapor increases the \log_{10} reduction rate of influenza viruses by a factor of 16 relative to the natural inactivation rate. Nevertheless, the disinfection rate for air saturated with TEG vapor at 25 to 29°C, which was measured



Fig 3. Natural inactivation of influenza viruses on stainless steel coupons at ambient conditions. Error bars correspond to \pm 1 SD based on tests using multiple coupons.

as 1.3 \log_{10} 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, the disinfection rate was 0.33 \log_{10} reductions for TEG vapor, compared with 3.6 \log_{10} reductions for HP vapor. Nonetheless, TEG vapor has some important advantages for use as a disinfectant.

For surface disinfection using TEG vapor, ambient air or warmed air could be easily saturated with TEG before being introduced into a space. Alternatively, micrometer-size TEG droplets, which evaporate rapidly, could be injected into the supply air duct or directly into the space. The standard method for introducing TEG droplets into the air for the purpose of air disinfection is through the use of a pressurized liquid,¹² although a nebulizer could be used as well. If a pandemic were to occur, both surface and air disinfection could take place simultaneously even while people occupied the space. Although an objection could be raised due to the potential health risk of using TEG vapor for air disinfection, this concern is likely unwarranted, because TEG is an odorless chemical of no known toxicity, and human exposure 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.²⁰ In addition, products containing TEG packaged in aerosol cans and designed to be sprayed into the air inside homes to control odors (eg, Oust, Febreze) are sold in stores everywhere.

According to the EPA,¹² "the Agency has no risk concerns for triethylene glycol with respect to human

Table I. TEG concentration in	air
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Temperature, °C	Concentration, ppm
20	1.1
25	1.7
30	2.8
35	4.4
40	6.9

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.¹³ 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 its concentration becomes sufficiently high to be germicidal.^{21,22}

It is reasonable to expect that the efficacy of TEG vapor will increase as its concentration is increased. At 25°C, however, the concentration of TEG in air cannot exceed 1.7 ppm, because air is saturated at that concentration. The only way to increase concentration is to increase temperature. As shown in Table 1, which was calculated from eqs (1) and (2), modest increases in temperature result in significant increases in TEG vapor concentration. For example, the concentration is more than 4 times higher at 35°C (95°F) than at 25°C. Thus, further work investigating TEG vapor as a disinfecting agent is warranted. Specifically, the effectiveness of TEG vapor for surface disinfection at higher concentrations (ie, at temperatures above room temperature) should be investigated, and the influence of RH on the effectiveness of disinfection should be evaluated.

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