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TECHNICAL NOTE

Impact of test methodology on the efficacy of triethylene glycol (Grignard Pure) against bacteriophage MS2

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ABSTRACT

The COVID-19 pandemic has raised interest in using chemical air treatments as part of a strategy to reduce the risk of disease transmission, but more information is needed to characterize their efficacy at scales translatable to applied settings and to develop standardized test methods for characterizing the performance of these products. Grignard Pure, a triethylene glycol (TEG) active ingredient air treatment, was evaluated using two different test protocols in a large bioaerosol test chamber and observed to inactivate bacteriophage MS2 in air (up to 99.9% at 90 min) and on surfaces (up to 99% at 90 min) at a concentration of approximately 1.2–1.5 mg/m³. Introducing bioaerosol into a TEG-charged chamber led to overall greater reductions compared to when TEG was introduced into a bioaerosol-charged chamber, although the differences in efficacy against airborne MS2 were only significant in the first 15 min. Time-matched control conditions (no TEG present) and replicate tests for each condition were essential for characterizing treatment efficacy. These findings suggest that chemical air treatments could be effective in reducing the air and surface concentrations of infectious pathogens in occupied spaces, although standard methods are needed for evaluating their efficacy and comparing results across studies. The potential health impacts of chronic exposure to chemicals should also be considered, but those were not evaluated here.

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Introduction

The COVID-19 pandemic has raised awareness about the role of airborne disease transmission in the spread of respiratory viruses in indoor environments (Bourouiba 2021; Peng et al. 2022; Samet et al. 2021; Tang et al. 2021; Wang et al. 2021). Particularly in poorly ventilated spaces, virus-laden aerosols emitted by infectious individuals can concentrate and remain airborne for hours (Tang et al. 2021; Wang et al. 2021). Accordingly, there has been an increasing interest in technologies that can reduce pathogen concentrations in the air, either through particle capture or inactivation. Increasing ventilation and maximizing filtration are well-accepted methods for reducing the risk of disease transmission (Bueno de Mesquita et al. 2022; Fadaei 2021; Peng et al. 2022; Wang et al. 2021), but certain circumstances make these strategies challenging to implement, for example, due to cost,

security, or engineering reasons (Bueno de Mesquita et al. 2022; National Academies of Sciences 2020). In addition to ultraviolet-C (UV-C) irradiation (Blatchley et al. 2023; Eadie et al. 2022; Fischer et al. 2022; Park, Mistrick and Rim 2022), electronic air treatment devices (Baselga, Alba and Schuhmacher 2023; Bono et al. 2021; Ratliff et al. 2023; Zeng, Heidarinejad and Stephens 2022), and portable air cleaners (including those with high-efficiency particulate air [HEPA] filters) (Busing et al. 2022; Rodríguez et al. 2021; Rogak et al. 2022), the use of chemical air treatments in occupied spaces has also been considered (Desai et al. 2023; Gomez et al. 2022).

There is a long (ca. 80 year) history of research related to the germicidal properties of glycols in the air (Lester Jr et al. 1950; Puck 1947a; Puck 1947b; Robertson et al. 1941). Triethylene glycol (TEG), which has been commonly used in fog machines for producing theatrical smoke, has a history of

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demonstrated efficacy against certain airborne viruses, bacteria, and fungi (Lester Jr et al. 1950; Puck 1947a; Puck 1947b; Robertson et al. 1943; Rudnick et al. 2009). More recent research and testing motivated by the COVID-19 pandemic demonstrated TEG's efficacy against bioaerosols (Desai et al. 2023). TEG is most germicidal close to its saturation point, which can be reached at a relatively low concentration [saturation concentration $\sim 4 \text{ mg/m}^3$ at 25°C and 50% relative humidity (Wise and Puck 1947)] due to its low vapor pressure [0.178 Pa at 25°C (Ballantyne and Snellings 2007)] (Lester Jr et al. 1950; Puck 1947b; Rudnick et al. 2009). Increasing relative humidity both lowers the saturation concentration of TEG in the air (because of its water solubility) and lowers the equilibrium concentration of TEG in aerosols (because aerosols will contain a higher fraction of water in higher relative humidity settings), both of which would reduce its efficacy against bioaerosols (Lester Jr et al. 1950; Puck 1947b). TEG tends to condense on cool surfaces and particulate matter because of its low vapor pressure, which results in greater loss of TEG from the air (Lester Jr et al. 1950). While there are no occupational exposure limits for TEG in the United States, the German maximum workplace air concentration (MAK) is 1000 mg/m^3 , based on an 8-h time-weighted average exposure in a 40-h workweek (Deutsche Forschungsgemeinschaft 2020).

In the United States, the Environmental Protection Agency (EPA) has the responsibility of regulating pesticide products, including antimicrobial pesticides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The product registration process includes a comprehensive Agency review of the available safety and efficacy data for each product. Under FIFRA, all pesticidal products must be used in accordance with their label directions. Although standardized methods exist for evaluating the efficacy of pesticide products in suspension and on surfaces, there is a notable lack of standardized testing methods for products intended to be used against pathogens in the air. This is in part due to the variations and complexity in indoor environments, which vary in size, composition, and occupancy, and exhibit a wide range of airflow and environmental conditions. An understanding of how these factors might impact product efficacy is necessary for predicting performance in the real world and is important for developing useful and reliable standardized test methods.

In this study, the efficacy of Grignard Pure was evaluated against bacteriophage MS2 (a non-enveloped virus that infects *Escherichia coli*) in the air and on

surfaces in a large-scale test chamber using two different testing methodologies. As a Biosafety Level (BSL)-1 microorganism, MS2 is safer to use than pathogenic agents (e.g., SARS-CoV-2 is BSL-3), making it more suitable to use in routine bioaerosol research and standardized testing. As a small, non-enveloped virus, MS2 is expected to be more resistant to chemical inactivation than enveloped viruses, such as SARS-CoV-2 and influenza (Rutala and Weber 2014; Spaulding and Emmons 1958). In addition to control tests, where no product was dispersed in the chamber, two different product introduction sequences were evaluated to determine the impact of the test methods on the product's calculated performance. The first test scenario involved aerosolizing virus into the chamber first prior to introducing Grignard Pure. This allows for a direct assessment of Grignard Pure at the target concentration against a high concentration of MS2 as a function of time. The second scenario consisted of reaching the target concentration of the product in the test chamber and subsequently aerosolizing MS2 into the treated space, where it can immediately interact with the product. This was intended to be more representative of use of Grignard Pure in occupied spaces, where virus could be introduced by an infected individual in a space where the target concentration of Grignard Pure is maintained. An improved understanding of how the testing protocols impact product efficacy is useful for developing and establishing standardized test methods and product performance benchmarks, both of which are intended to inform how chemical air treatment products are evaluated and regulated.

Materials and methods

For a detailed description of the test chamber and microbiological methods, please see Ratliff et al. (2023). In short, experiments were conducted in a large chamber ($3.6 \text{ m} \times 7.6 \text{ m} \times 3.0 \text{ m}$, 85 m^3) at EPA's Aerosol Test Facility with a mock heating, ventilation, and air conditioning (HVAC) system (with no filter present) and mixing fans. Air is recirculated through the HVAC system using a negative air machine (Omni-Aire 1000 V, Omnitech, Mukilteo, WA), operating at 165 L/s, resulting in approximately 7 air changes per hour (100% recirculated air). Temperature and relative humidity (RH) in the chamber were regulated by a supervisory control and data acquisition (SCADA) system, and all experiments were conducted at $22 \pm 2^\circ\text{C}$ with an RH of 30-35%. In between experiments, the chamber was reset by opening the chamber and recirculating its air through

multiple high-efficiency particulate air filter banks until negligible particles were present based on measurements with an Aerodynamic Particle Sizer (Model 3321, TSI, Inc., Shoreview, MS, USA) and a Scanning Mobility Particle Sizer (Model 3080 Electrostatic Classifier/TSI 3010 Condensation Particle Counter, TSI, Inc.), and background samples were taken prior to the initiation of each test to ensure that no MS2 was present in the air prior to aerosolization.

In each experiment, the inoculum (target concentration 10^{10} plaque-forming unit [PFU]/mL) was prepared by diluting 8 mL of MS2 stock (ATCC 15597-B1) into 32 mL of filter-sterilized deionized water and aerosolized using four 6-jet Collison Nebulizers (part no. ARG CNB3, CH Technologies, Westwood, NJ, USA) over a 10-minute period immediately prior to the first bioaerosol sampling period (time = 0 min sample). SKC BioSamplers (SKC Inc., Eighty-Four, PA, USA) containing 20 mL of $1\times$ phosphate-buffered saline (PBS) (P0196, Teknova, Hollister, CA, USA) were used to collect air samples during testing, drawing air for 10 min during each sampling period at 12.5 L/min from two locations at “breathing zone” height within the chamber. Herein, the sample time is noted by the start of each 10-minute sampling period, where time = 0 min represents the time at which the bioaerosol has been fully introduced into the test chamber (i.e., the end of the aerosolization period). MS2 stock was prepared using a top agar overlay technique (Kropinski et al. 2009), and microbiological test samples were stored on ice or refrigerated at 4°C and plated within two hours following the completion of each test using a plaque assay with the host *E. coli* (ATCC 15597) following various dilutions and the same conventional overlay method. After an overnight incubation at $35\pm 2^{\circ}\text{C}$, plaques were manually enumerated the following day.

Neutralization tests were performed with TEG and the collection media prior to the aerosol testing to ensure that no additional inactivation of MS2 occurred after the bioaerosol samples were collected. In addition to an inoculation control, where no Grignard Pure was added, two conditions were tested; in the first, 100 μL of Grignard Pure was added to 20 mL of $1\times$ PBS already inoculated with MS2 at a target concentration of $\sim 10^5$ PFU/sample, and in the second, MS2 was inoculated following the addition of 100 μL of Grignard Pure. Under both conditions, samples were stored for 2 h at 4°C and subsequently plated and enumerated. Because this concentration is substantially higher than the amount of Grignard Pure that would be collected in each impinger during

the sampling period and no reduction in MS2 recoveries were observed between the inoculation controls and the neutralization test samples, no additional neutralizer was used.

Small-scale surface samples were also used to evaluate the impact of Grignard Pure on bioaerosol settling and deposition processes (i.e., if the product impacted aerosol agglomeration), as well as efficacy of the product in the air against MS2 on surfaces. Uniform pieces of 2 cm x 4 cm stainless steel material (coupons) were cleaned, rinsed, and sterilized prior to use. Inactivation coupons were inoculated at a target concentration of 10^7 PFU/coupon, whereas deposition coupons were left blank (i.e., not inoculated prior to testing). The two sets of coupons were placed collocated at five different locations on the chamber floor (see Figure 1, Ratliff et al. [2023]). Following each test, both sets of coupons were extracted in PBS and enumerated *via* plaque assay.

Grignard Pure was dispersed into the test chamber by connecting a theatrical haze machine (AmHaze Stadium, CHAUVET Professional, Sunrise, FL) to a 14” x 14” section of the recirculating mock HVAC system (downstream of the blower) *via* a 4” galvanized duct. The amount of product present in the test

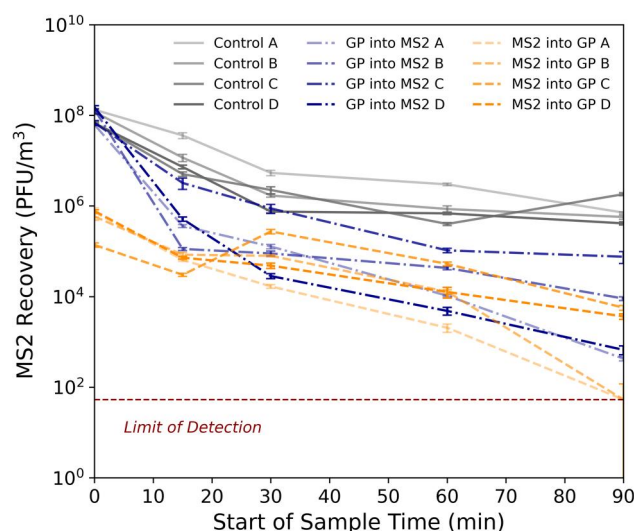


Figure 1. MS2 recoveries at each sampling time point during the Grignard Pure and control tests, where “GP into MS2” represents the test condition where the product is introduced into the chamber following aerosolization of MS2, and “MS2 into GP” represents the bioaerosol being introduced into the chamber with the product at its target concentration. Each data point represents an average recovery over duplicate bioaerosol samples as determined by plaque assay during each respective test (A-D), and error bars represent pooled standard deviation of triplicate plating and duplicate bioaerosol samples at each sample time point. Time = 0 min represents the end of the MS2 aerosolization period and the beginning of the first bioaerosol sampling window.

chamber was monitored by DustTrak aerosol monitors (DRX 8534, TSI Inc., Shoreview, MN, USA) following a correlation curve developed by the product manufacturer (Desai et al. 2023). The concentration of Grignard Pure was controlled by adjusting the output of the haze machine and manually cycling it on for 5 s at a time at various intervals (averaging 3 min) that were determined by monitoring the concentration *via* the aerosol monitors. The objective was to maintain a time-weighted average (TWA) aerosol concentration between 0.1 mg/m^3 and 0.5 mg/m^3 in the chamber throughout the duration of the tests. The average concentration of TEG in the chamber was also measured during three different tests (one GP into MS2 test and two MS2 into GP tests) in accordance with the National Institute for Occupational Safety and Health (NIOSH) Method 5523 and determined to be $1.2\text{--}1.5 \text{ mg/m}^3$ during testing. In this method, a known volume of air is pulled through XAD-7 OVS sorbent tubes (#226-57, SKC Inc.) containing a glass fiber filter. Methanol is added to the tubes to desorb the analyte from both the sorbent and the glass fiber filter, and the eluent is analyzed using gas chromatography with a flame ionization detector. The aerosol monitors and samples collected for NIOSH Method 5523 were collocated with the bioaerosol sampling locations. This technology was selected for evaluation based on stakeholder interest and was provided to EPA through a materials transfer agreement.

Two different test scenarios were used to evaluate the efficacy of Grignard Pure against MS2. In the first scenario, MS2 was aerosolized into the test chamber, an initial bioaerosol test sample was taken (time = 0 min), and then the Grignard Pure product was introduced into the chamber ("GP into MS2"). In the second scenario, the Grignard Pure product was introduced into the chamber at its target concentration before MS2 aerosolization and the initial bioaerosol test sample was collected ("MS2 into GP"). Four replicates of each test scenario (plus a control scenario, with no Grignard Pure introduced) were conducted.

To quantify efficacy, $\log_{10}$ reduction was calculated as the difference between the mean MS2 recoveries from control tests and the Grignard Pure tests at time-matched sampling points or by comparing recoveries from control coupons to test coupons. Python (version 3.9.12), including packages NumPy (version 1.23.4) and pandas (version 1.5.1), was used for statistical calculations. Differences in group medians for each test type at each sampling time point were evaluated using Wilcoxon rank sum tests, and for data that satisfied the normality assumption (following a

normality check using Shapiro-Wilk tests on $\log_{10}$ -transformed data), differences in group means were also evaluated using Welch's t-tests, with significance determined at the $\alpha = 0.05$ level.

Results and discussion

The amount of MS2 recovered (in PFU/ m^3) during each sampling time point during all tests is shown in Figure 1, and Figure 2a shows the average concentration of MS2 over time for each Grignard Pure test scenario and the control tests. The corresponding $\log_{10}$ reductions, comparing each respective Grignard Pure test scenario to the controls, are shown in Figure 2b. When the product was present, bioaerosol concentrations were always significantly lower than

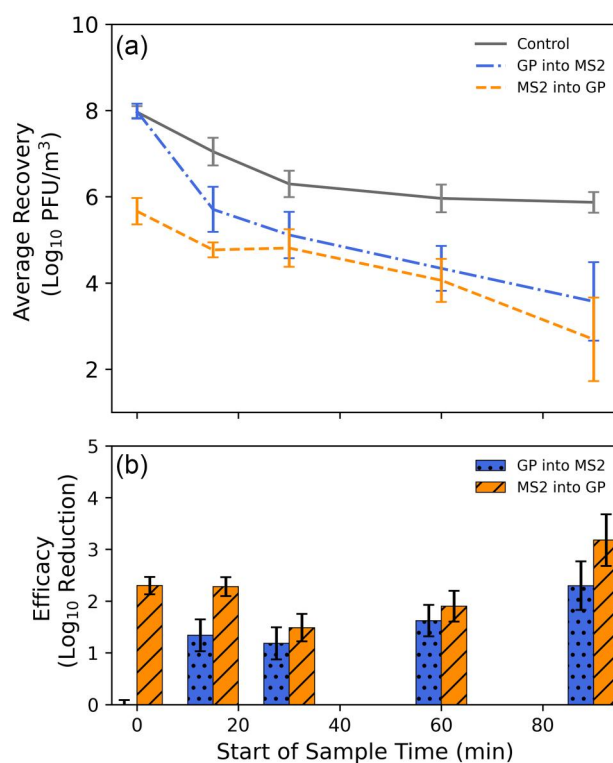


Figure 2. Results from Grignard Pure efficacy tests, where "GP into MS2" represents the test condition where the product was introduced into a high bioaerosol concentration, and "MS2 into GP" represents the test condition where bioaerosol was introduced into the Grignard Pure product at its target concentration. (a) Average $\log_{10}$ recovery of bacteriophage MS2 at each sampling time point during the control and product tests, where error bars represent the standard deviation in $\log_{10}$ recoveries in each set of tests ($n = 4$). (b) Efficacy of Grignard Pure, calculated as the $\log_{10}$ reduction for each type of test compared to the control tests. Error bars represent pooled standard error from both the control and Grignard Pure tests. Time = 0 min represents the end of the MS2 aerosolization period and the beginning of the first bioaerosol sampling window.

control conditions (Table 1). The maximum $\log_{10}$ reductions for both test scenarios occur at the final (90 min) sampling time point, achieving 2.3 $\log_{10}$ (99.5%) and 3.2 $\log_{10}$ (99.9%) reductions for the GP into MS2 and MS2 into GP scenarios, respectively.

Comparing results from the two different test scenarios where the order of virus and product introduction is switched is insightful for understanding how study design can impact calculated efficacy against airborne MS2, as well as how the product might perform in an occupied space with active viral shedders. Aerosolizing MS2 into the test chamber with the product already present reduced the initial sample by 2.3 $\log_{10}$ (99.5%) due to the presence of the product. The 15-min samples differed by 0.9 $\log_{10}$ (88%) resulting from the differences in product contact time between the two test scenarios. Accordingly, there are significant differences in comparing recoveries from the two product test types at each of these sampling time points (both time = 0 and 15 min, Table 1). Although the calculated log reductions for the MS2 into GP test scenario are consistently higher than the GP into MS2 scenario, the differences in recoveries between the two test conditions at the other sampling time points (time = 30, 60, 90 min) are not statistically significant ($p > 0.05$). This suggests that, under these test conditions, changing the order of virus and product introduction impacts efficacy early during the test period (because of the notable differences in the product contact time), but it ultimately does not significantly impact the later portions of the test period,

when the calculated $\log_{10}$ reductions are highest and the differences between the contact times are not as great.

Consistent with the findings of Ratliff et al. (2023), the results from these experiments underscore the importance of replicate testing that includes time-matched controls for characterizing air treatment efficacy. For both Grignard Pure test scenarios, MS2 recoveries range up to $>2 \log_{10}$ PFU/m³ at a specific sampling time point, with the greatest range in recoveries for both scenarios at time = 90 min (Figure 1). Although there is less variability in the control tests, the differences in recoveries still range up to nearly 1 $\log_{10}$ PFU/m³ (0.85 and 0.87 $\log_{10}$ PFU/m³ for time = 15 and 60 min, respectively, Figure 1). Averaging over the set of control experiments, attenuation due to natural decay, wall losses, and settling account for approximately 2.1 $\log_{10}$ PFU/m³ reduction in MS2 recoveries over the duration of the 90-min test period (Figure 2a). Accordingly, under these test conditions, calculating air treatment efficacy by comparing the concentrations during the product test time points to the initial bioaerosol load (instead of time-matched control conditions that account for background bioaerosol decay not attributed to the product) can overstate the product efficacy by over 99%.

Two different sets of surface samples were included in the experiments presented here: deposition (blank) coupons, which were intended to evaluate how much viable MS2 was deposited on surfaces during each test, and inactivation (inoculated) coupons, which

Table 1. Summary of calculated $\log_{10}$ reductions and p-values from Welch's t-tests (for data that passed a formal normality check; otherwise, a dash represents that the assumption was not met) and Wilcoxon Rank Sum tests.

Sample Type	Test Comparison	Time (min)	$\log_{10}$ Reduction Between Tests	Welch's T-Test p-value	Rank Sum Test p-value
Air	Control vs. GP into MS2	0	-0.02	0.88	0.56
		15	1.34	0.013	0.021
		30	1.18	0.022	0.043
		60	1.62	0.0058	0.021
		90	2.30	0.019	0.021
	Control vs. MS2 into GP	0	2.30	—	0.021
		15	2.28	0.00020	0.021
		30	1.49	0.0039	0.021
		60	1.90	0.0025	0.021
		90	3.18	0.0086	0.021
	GP into MS2 vs. MS2 into GP	0	2.32	—	0.021
		15	0.94	0.047	0.021
		30	0.30	0.48	0.39
		60	0.28	0.53	0.77
		90	0.88	0.30	0.25
Surface - Deposition	GP into MS2 vs. Control	n/a	n/a	—	0.00015
	MS2 into GP vs. Control	n/a	n/a	—	0.00001
	GP into MS2 vs. MS2 into GP	n/a	n/a	—	0.30
Surface - Inoculation	Control vs. GP into MS2	n/a	1.35	2.3E-08	4.1E-06
	Control vs. MS2 into GP	n/a	1.88	1.5E-11	3.8E-06
	GP into MS2 vs. MS2 into GP	n/a	n/a	0.011	0.012

Where $\log_{10}$ reductions are shown, the calculated value is based on the difference in average recoveries from the Test Comparison column (calculated by subtracting recoveries from the second stated test in the Test Comparison column from the first). A value of "n/a" means that value is either not relevant or not meaningful. Bold text represents a statistically significant difference ($\alpha < 0.5$) between paired data.

were included to evaluate the efficacy of Grignard Pure against MS2 on surfaces. The MS2 recoveries from both sets of coupons are shown in Figure 3. Across all coupon and test types, MS2 recoveries from the control tests were consistently higher than either Grignard Pure test scenario, and recoveries were generally lowest when MS2 was introduced into the chamber with the product already present (consistent with the lower initial bioaerosol concentration for this test condition, Figures 1 and 2). Pooling across coupon locations respective of each test type, differences in recoveries from both product test scenarios compared to both types of control test coupons were statistically significant (Table 1). Averaging across coupon locations and replicate tests, the $\log_{10}$ reduction of the inactivation coupons (compared to recoveries from the control tests) was 1.4 for the GP into MS2 scenario and 1.9 for the MS2 into GP scenario (Table 1). Comparing recoveries between the two Grignard Pure test scenarios, only the inoculation coupons showed statistically significant differences between test types (i.e., deposition coupon recoveries from the two types of tests with the product present were not statistically different). Because inactivation was evident on the coupons directly inoculated with MS2 (Figure 3b), it is likely that some of the MS2 that settled on the deposition coupons was also inactivated by the presence of the product in the air.

It is critical to consider challenges that exist in extrapolating results from any laboratory-based testing to predicting product performance in applied settings. In this study, calculated efficacy is a function of the test conditions. The response of respiratory pathogens such as SARS-CoV-2, influenza, and *Mycobacterium*

tuberculosis to TEG will differ from the test microorganism used in this study (bacteriophage MS2) (Rutala and Weber 2014; Spaulding and Emmons 1958). Changing aerosolization and sampling methods could also impact efficacy, as could airflow and environmental conditions in the test chamber (including temperature and relative humidity). The real-world conditions under which air treatment products could be used vary widely, with differing HVAC configurations and airflow patterns, a variety of surfaces (e.g., porous) and objects in a space, and diversity in other particles and chemistry in the air, all of which could impact the interaction between the air treatment and target pathogen. Moreover, controlling the concentration of air treatments in indoor spaces could present a challenge under certain conditions; in this study, the measured TEG concentration was greater than twice the maximum of the targeted average aerosol concentration.

The results presented herein demonstrate that Grignard Pure, an antimicrobial air treatment with TEG as the active ingredient, can inactivate airborne MS2 (up to 99.9% in 90 min) and MS2 on surfaces (up to 99% after 90 min) under these test conditions. Introducing the virus into the test chamber with the product already at its target concentration led to consistently lower bioaerosol recoveries than when the product was introduced into a high bioaerosol concentration, but the differences between these two methodologies did not lead to statistically significant differences in efficacy against bioaerosols after 15 min. Time-matched controls and replicate tests for each condition were necessary for characterizing efficacy. The results presented herein demonstrate that changes

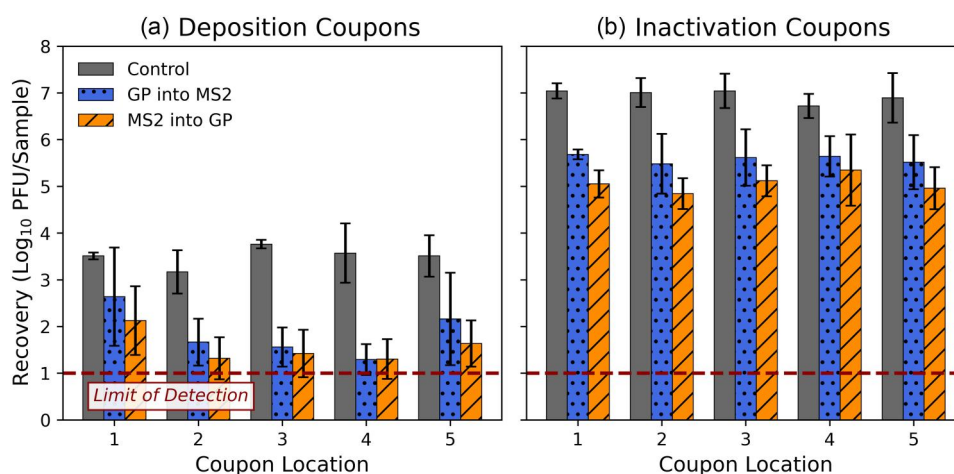


Figure 3. $\log_{10}$ recoveries of MS2 from surface samples (stainless steel coupons) following tests, averaged over each test type for each coupon location. (a) The amount of viable MS2 deposited on and recovered from coupons initially inserted into the test chamber blank. (b) The amount of MS2 recovered from coupons that were inoculated with MS2 prior to placing them in the chamber for each test. Error bars represent standard deviation in recoveries for each test type ($n = 4$).

in test protocols can affect calculated air treatment efficacy, so caution should be exercised in juxtaposing results from different studies, and standardized testing is needed to compare findings more effectively.

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Disclaimer

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are openly available at <https://doi.org/10.23719/1528421>.

References

- Ballantyne, B., and W. M. Snellings. 2007. Triethylene glycol ho(ch₂ch₂o)₃h. *J. Appl. Toxicol.* 27 (3):291–9. doi:10.1002/jat.1220.
- Baselga, M., J. J. Alba, and A. J. Schuhmacher. 2023. Impact of needle-point bipolar ionization system in the reduction of bioaerosols in collective transport. *Sci. Total Environ.* 855:158965. doi:10.1016/j.scitotenv.2022.158965.
- Blatchley, E. R., D. J. Brenner, H. Claus, T. E. Cowan, K. G. Linden, Y. Liu, T. Mao, S.-J. Park, P. J. Piper, R. M. Simons, et al. 2023. Far UV-c radiation: An emerging tool for pandemic control. *Crit. Rev. Environ. Sci. Technol.* 53 (6):733–53. doi:10.1080/10643389.2022.2084315.
- Bono, N., F. Ponti, C. Punta, and G. Candiani. 2021. Effect of UV irradiation and TiO₂-photocatalysis on airborne bacteria and viruses: An overview. *Materials* 14 (5):1075. doi:10.3390/ma14051075.
- Bourouiba, L. 2021. The fluid dynamics of disease transmission. *Annu. Rev. Fluid Mech.* 53 (1):473–508. doi:10.1146/annurev-fluid-060220-113712.
- Bueno de Mesquita, P. J., W. W. Delp, W. R. Chan, W. P. Bahnfleth, and B. C. Singer. 2022. Control of airborne infectious disease in buildings: Evidence and research priorities. *Indoor Air* 32 (1):e12965. doi:10.1111/ina.12965.
- Buising, K. L., R. Schofield, L. Irving, M. Keywood, A. Stevens, N. Keogh, G. Skidmore, I. Wadlow, K. Kevin, B. Rismanchi, et al. 2022. Use of portable air cleaners to reduce aerosol transmission on a hospital coronavirus disease 2019 (COVID-19) ward. *Infect. Control Hosp. Epidemiol.* 43 (8):987–92. doi:10.1017/ice.2021.284.
- Desai, G., G. Ramachandran, E. Goldman, W. Esposito, A. Galione, A. Lal, T. K. Choueiri, A. Fay, W. Jordan, D. W. Schaffner, et al. 2023. Efficacy of grignard pure to inactivate airborne phage ms2, a common SARS-CoV-2 surrogate. *Environ. Sci. Technol.* 57 (10):4231–40. doi:10.1021/acs.est.2c08632.
- Eadie, E., W. Hiwar, L. Fletcher, E. Tidswell, P. O'Mahoney, M. Buonanno, D. Welch, C. S. Adamson, D. J. Brenner, C. Noakes, et al. 2022. Far-UVC (222 nm) efficiently inactivates an airborne pathogen in a room-sized chamber. *Sci. Rep.* 12 (1):4373. doi:10.1038/s41598-022-08462-z.
- Fadaei, A. 2021. Ventilation systems and COVID-19 spread: Evidence from a systematic review study. *Eur. J. Sustainable Dev. Res.* 5 (2):em0157. doi:10.21601/ejodsr/10845.
- Fischer, R. J., J. R. Port, M. G. Holbrook, K. C. Yinda, M. Creusen, J. ter Stege, M. de Samber, and V. J. Munster. 2022. UV-c light completely blocks aerosol transmission of highly contagious SARS-CoV-2 variants wu1 and delta in hamsters. *Environ. Sci. Technol.* 56 (17):12424–30. doi:10.1021/acs.est.2c02822.
- Forschungsgemeinschaft, D. 2020. List of substances. In *List of MAK and BAT values*, vol. 2019, 20–138. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.
- Gomez, O., K. M. McCabe, E. Biesiada, B. Volbers, E. Kraus, M. N. Caballero, and M. Hernandez. 2022. Airborne murine coronavirus response to low levels of hypochlorous acid, hydrogen peroxide and glycol vapors. *Aerosol Sci. Technol.* 56 (11):1047–57. doi:10.1080/02786826.2022.2120794.
- Kropinski, A. M., A. Mazzocco, T. E. Waddell, E. Lingohr, and R. P. Johnson. 2009. Enumeration of bacteriophages by double agar overlay plaque assay. In *Bacteriophages: Methods and protocols, volume 1: Isolation, characterization, and interactions*, ed. M. R. J. Clokie and A. M. Kropinski, 69–76. Totowa, NJ: Humana Press.
- Lester, W. Jr, S. Kaye, O. Robertson, and E. W. Dunklin. 1950. Factors of importance in the use of triethylene glycol vapor for aerial disinfection. *Am. J. Public Health Nations. Health* 40 (7):813–20. doi:10.2105/ajph.40.7.813.
- National Academies of Sciences, Engineering, and Medicine. 2020. The national academies collection: Reports funded by National Institutes of Health. In *Airborne Transmission of SARS-CoV-2: Proceedings of a*

- Workshop—in Brief*, ed. A. Staudt, J. Saunders, J. Pavlin, and M. Shelton-Davenport. Washington, DC: National Academies Press (US).
- Park, S., R. Mistrick, and D. Rim. 2022. Performance of upper-room ultraviolet germicidal irradiation (UVGI) system in learning environments: Effects of ventilation rate, UV fluence rate, and UV radiating volume. *Sustainable Cities Soc.* 85:104048. doi:10.1016/j.scs.2022.104048.
- Peng, Z., A. L. P. Rojas, E. Kropff, W. Bahnfleth, G. Buonanno, S. J. Dancer, J. Kurnitski, Y. Li, M. G. L. C. Loomans, L. C. Marr, et al. 2022. Practical indicators for risk of airborne transmission in shared indoor environments and their application to COVID-19 outbreaks. *Environ. Sci. Technol.* 56 (2):1125–37. doi:10.1021/acs.est.1c06531.
- 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 (6):729–39. doi:10.1084/jem.85.6.729.
- 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 (6):741–57. doi:10.1084/jem.85.6.741.
- Ratliff, K. M., L. Oudejans, J. Archer, W. Calfee, J. U. Gilberry, D. A. Hook, W. E. Schoppman, R. W. Yaga, L. Brooks, and S. Ryan. 2023. Large-scale evaluation of microorganism inactivation by bipolar ionization and photocatalytic devices. *Build. Environ.* 227:109804. doi:10.1016/j.buildenv.2022.109804.
- Robertson, O. H., E. Bigg, B. F. Miller, and Z. Baker. 1941. Sterilization of air by certain glycols employed as aerosols. *Science* 93 (2409):213–4. doi:10.1126/science.93.2409.213.
- Robertson, O., T. T. Puck, H. F. Lemon, and C. G. Loosli. 1943. The lethal effect of triethylene glycol vapor on airborne bacteria and influenza virus. *Science* 97 (2510):142–4. doi:10.1126/science.97.2510.142-b.
- Rodríguez, M., M. L. Palop, S. Seseña, and A. Rodríguez. 2021. Are the portable air cleaners (pac) really effective to terminate airborne SARS-CoV-2? *Sci. Total Environ.* 785:147300. doi:10.1016/j.scitotenv.2021.147300.
- Rogak, S. N., A. Rysanek, J. M. Lee, S. V. Dhulipala, N. Zimmerman, M. Wright, and M. Weimer. 2022. The effect of air purifiers and curtains on aerosol dispersion and removal in multi-patient hospital rooms. *Indoor Air* 32 (10):e13110. doi:10.1111/ina.13110.
- Rudnick, S. N., J. J. McDevitt, M. W. First, and J. D. Spengler. 2009. Inactivating influenza viruses on surfaces using hydrogen peroxide or triethylene glycol at low vapor concentrations. *Am. J. Infect. Control.* 37 (10):813–9. doi:10.1016/j.ajic.2009.06.007.
- Rutala, W. A., and D. J. Weber. 2014. Selection of the ideal disinfectant. *Infect. Control Hosp. Epidemiol.* 35 (7):855–65. doi:10.1086/676877.
- Samet, J. M., K. Prather, G. Benjamin, S. Lakdawala, J.-M. Lowe, A. Reingold, J. Volckens, and L. C. Marr. 2021. Airborne transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): What we know. *Clin. Infect. Dis.* 73 (10):1924–6. doi:10.1093/cid/ciab039.
- Spaulding, E. H., and E. K. Emmons. 1958. Chemical disinfection. *Am. J. Nurs.* 58 (9):1238–42. doi:10.2307/3472880.
- Tang, J. W., W. P. Bahnfleth, P. M. Bluyssen, G. Buonanno, J. L. Jimenez, J. Kurnitski, Y. Li, S. Miller, C. Sekhar, L. Morawska, et al. 2021. Dismantling myths on the airborne transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J. Hosp. Infect.* 110:89–96. doi:10.1016/j.jhin.2020.12.022.
- Wang, C. C., K. A. Prather, J. Sznitman, J. L. Jimenez, S. S. Lakdawala, Z. Tufekci, and L. C. Marr. 2021. Airborne transmission of respiratory viruses. *Science* 373 (6558):eabd9149. doi:10.1126/science.abd9149.
- Wise, H., and T. T. J. S. Puck. 1947. Saturation concentrations of triethylene glycol vapor at various relative humidities and temperatures. *Science* 105 (2734):556–7. doi:10.1126/science.105.2734.556.
- Zeng, Y., M. Heidarinejad, and B. Stephens. 2022. Evaluation of an in-duct bipolar ionization device on particulate matter and gas-phase constituents in a large test chamber. *Build. Environ.* 213:108858. doi:10.1016/j.buildenv.2022.108858.