OZONE AND GLYCOL VAPOR DECONTAMINATION OF AIR IN A CLOSED ROOM

G. B. Pelleu, Jr., et al

Naval Graduate Dental School
Bethesda, Maryland

1 July 1973

Security Classification

AD - 774526

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Naval Graduate Dental School
National Naval Medical Center
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b GROUP

3. REPORT TITLE
OZONE AND GLYCOL VAPOR DECONTAMINATION OF AIR IN A CLOSED ROOM

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(Last name, first name, initial)*
G. B. Pelleu, Jr., Ph.D.
DT2 R. F. Berry, USN
DT2 N. G. Holleman, USN

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
1 July 1973	214	25

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	NGDS-TR-024
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d. WORK UNIT NO. MR041.20.02-6050B3II	

10. [illegible] DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	BUREAU OF MEDICINE AND SURGERY Navy Department Washington, D. C. 20390

13. ABSTRACT

A dielectric type generator (OzoneAir) and two commercial glycol-type spray decontaminants (Ozium and Air-Fresh) were evaluated in a closed room for effectiveness in reducing the number of airborne bacteria. Ozone in concentrations of 0.05, 0.1 (the threshold limit value for humans), and 1.0 p.p.m., and the two commercial glycol aerosols, were tested in a 700-ft.^{3} capacity closed room for their effects on reductions in the number of airborne Streptococcus mitis, Staphylococcus epidermidis, and Bacillus subtilis spores. At ozone concentrations of 1.0 p.p.m., more than 90 percent of the streptococci and staphylococci were removed from the air within 5 minutes. No airborne reductions were noted at the TLV (threshold limit value) concentration of ozone. The effect of the glycol aerosols on 60-minute reductions of airborne bacteria was no different from that of the water aerosol controls.

i

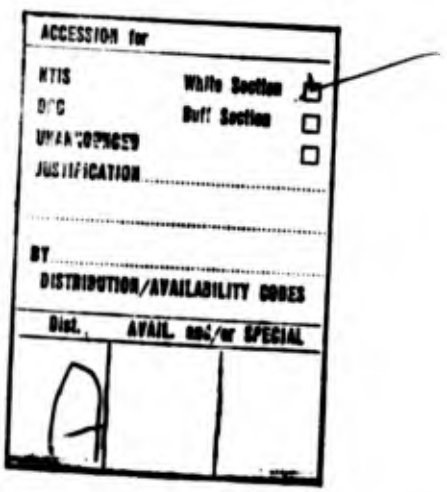
DD FORM 1 JAN 64 1473

UNCLASSIFIED
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Airborne Bacteria Decontamination of air Dental Operating Room Glycol vapor Ozone						

ADMINISTRATIVE INFORMATION

This work was accomplished under Bureau of Medicine and Surgery Research Work Unit MR041.20.02-6050B3II, and was supported through funds provided by the Bureau of Medicine and Surgery.



Submitted by:

G. B. PELLEU, JR., Ph. D.
Research Director and
Acting Chairman, Research Department

Approved by:

G. D. SELFRIDGE
Rear Admiral, DC, USN
Commanding Officer

ABSTRACT

A dielectric type generator (OzoneAir) and two commercial glycol-type spray decontaminants (Ozium and Air-Fresh) were evaluated in a closed room for effectiveness in reducing the number of airborne bacteria. Ozone in concentrations of 0.05, 0.1 (the threshold limit value for humans), and 1.0 p.p.m., and the two commercial glycol aerosols, were tested in a 700-ft.^{3} capacity closed room for their effects on reductions in the number of airborne Streptococcus mitis, Staphylococcus epidermidis, and Bacillus subtilis spores. At ozone concentrations of 1.0 p.p.m., more than 90 percent of the streptococci and staphylococci were removed from the air within 5 minutes. No airborne reductions were noted at the TLV (threshold limit value) concentration of ozone. The effect of the glycol aerosols on 60-minute reductions of airborne bacteria was no different from that of the water aerosol controls.

INTRODUCTION

Recognition of the probability that airborne contamination and cross infection are related has stimulated the development of many techniques for decontaminating the air of dental operating rooms (DOR's). The introduction of commercial dielectric-type ozone generators and aerosol disinfectant sprays has revived interest in ozone and glycols as a means of reducing the concentration of airborne bacteria. That ozone and glycol vapors have a bactericidal effect on airborne bacteria has been a well-established fact for some time.[1-7]

Reports of studies concerning the value of ozone for decontaminating air are conflicting and controversial. Some investigators report only minimal effects with ozone at concentrations above 1 0 p.p.m.,[8] whereas others report good bactericidal activity at concentrations as low as 0.025 p.p.m.[2] However, ozone concentrations in a clinical environment should not exceed a threshold limit value (TLV) of 0.1 p.p.m.[9] Levels at 1.0 p.p.m. are toxic for man and animals.[10]

Glycol vapor, on the other hand, is considered nontoxic[7,11] and has been shown to reduce incidence rates of respiratory infections in clinical studies.[12-14] Glycol aerosols must be in the vapor form, rather than in the mist form, to be germicidal. Glycol vapor in concentrations as small as 2 to 5 micrograms per liter of air rapidly kills many kinds of airborne bacteria. Several new types of aerosol sprays containing triethylene and propylene glycols are commercially available. These sprays are mixed with other active and inert ingredients, and are aerosolized by means of propellants, such as freon. It is not known, however, whether these glycol mixtures vaporize in concentrations sufficiently high to decontaminate air. The purpose of this study was to evaluate, in a closed room simulating a DOR, the effectiveness of a dielectric ozone generator and two commercial glycol disinfectant sprays for reducing the number of airborne microorganisms.

MATERIALS AND METHODS

All tests were conducted in a 700-cubic-foot capacity experimental room specially constructed for aerosol studies.[15] The relative humidity within the room was maintained between 30 and 40 percent with an ambient temperature of about 75° F.

DETERMINING THE EFFECT OF OZONE ON AIRBORNE BACTERIA.--Overnight broth cultures of Streptococcus mitis,* Staphylococcus epidermidis,* and a suspension of Bacillus subtilis spores† were diluted in sterile demineralized water and aerosolized into the room with a glass nebulizer‡ to a final concentration of about 175 viable particles per cubic foot of air (VP/ft.^{3}). A 15-inch oscillating fan was operated during tests to facilitate mixing of

*Isolated from the air of a DOR.
†Obtained from pilot plant at Fort Detrick, Md.
‡DeVilbiss No. 40, DeVilbiss Co., Somerset, Pa.

bacteria in the air of the room. Previous tests with an Andersen sampler* had shown the particle sizes of the three airborne bacteria to be within a 1- to 5-micron range.

Tests for the effect of ozone on airborne microorganisms were conducted for 2 consecutive hours in the room. Airborne microorganisms were aerosolized and allowed to settle normally for the first 1-hour period (control) and recoveries were made over 5-minute intervals, each beginning at 0, 15, 30, and 50 minutes after aerosolization. Ozone was introduced into the room during the second hour and bacteria were reaerosolized. Recoveries made at the same time intervals were then compared with those made during the first hour without ozone in the room. A dielectric ozone generator† was used to produce ozone concentrations of 0.05, 0.1 and 1.0 p.p.m. in the room. Ozone concentrations were constantly monitored during the test with a Mast ozone meter.‡ Before each 1-hour test period, the air in the room was cleaned for 20 minutes with a high efficiency particulate air (HEPA) filter module.§

Airborne bacteria were recovered with a 1-hour Reyniers Sampler** adjusted to draw air at a rate of 1 cubic foot per minute. The culture medium, in 150 X 25 mm. disposable plastic petri dishes, was prepared by layering 5 percent whole defibrinated rabbit blood in Trypticase†† soy agar on a previously poured layer of the same medium without blood. The plates were incubated at 37° C. for 24 hours and the colonies were counted in reflected light. Each colony was assumed to represent a single viable particle and the microbial concentration was defined as the number of viable particles per cubic foot of air. The plates were replaced at each time interval to avoid excessive ozone exposures to previously collected bacteria.

Tests were made to determine whether the culture medium would retain enough ozone to inhibit the growth of bacteria. Uninoculated plates were exposed in an activated Reyniers sampler to concentrations of 1.0 p.p.m. ozone for 5 minutes. A total of 0.05 ml. bacterial suspension, containing approximately 200 colony-forming units (c.f.u.), was spread until dry on the agar surface in areas of the plate corresponding to the four time positions. These counts were then compared with those of plates which had not been exposed to ozone.

DETERMINING THE EFFECT OF OZONE ON SURFACE AREAS.--Each of the three bacteria was aerosolized separately into the rooms as before and allowed to settle normally on a 12- X 14-inch formica-covered board. Microbial concentrations of 8 to 95 c.f.u. per square inch of surface area were exposed for 1 hour to 0.1 p.p.m. ozone. For comparison, this procedure was preceded by identical testing where no ozone was used. The surface

---

*Model No. 0101, Andersen Samplers & Consulting Service, Provo, Utah.
†OzoneAir Model AT200, Air and Water Purification Inc., Akron, Ohio.
‡Mast Model 724-21, Mast Development Co., Davenport, Iowa.
§Model No. 43, Agnew-Higgins, Garden Grove, Calif.
**Reyniers Sampler, Model FD-100, Reyniers and Son, Chicago, Ill.
††Baltimore Biological Laboratories, Inc., Baltimore, Md.

areas, equally divided into nine rectangular spaces, were randomly assayed for colony forming units, using the Rodac* impression plate technique.[16]

DETERMINING THE EFFECT OF DISINFECTANT SPRAYS ON AIRBORNE BACTERIA.-- Two commercially available glycol disinfectant sprays were used: The first, Ozium,† contained a total of 8.8 percent propylene and triethylene glycol, and the second, Air-Fresh,‡ contained propylene and triethylene glycol in unspecified amounts.

Tests for the effect of disinfectant sprays on the three airborne bacteria were carried out as before for 2 consecutive hours. Bacterial suspensions were aerosolized into the room as previously described, but this time with initial airborne concentrations of about 1.0×10^5 VP/ft.^{3} For the first hour, sterile distilled water was aerosolized from a spray can to simulate use of a disinfectant spray. For the second hour, bacterial aerosolization was immediately followed by spraying with either 3 grams of Ozium disinfectant§ or 9 grams of Air-Fresh disinfectant. These amounts were in excess of manufacturers' recommendations. The fan was also operated during tests to facilitate mixing of airborne bacteria with disinfectant. The air in the room was cleaned before each test by HEPA filtration and the exhaust fan was operated for 1 hour after each day's experiment, to remove any residual aerosol disinfectant.

All-glass liquid impingers containing 100 ml. of sterile water were used in testing glycol disinfectants, because preliminary tests showed that bacteria were inhibited from growing on agar plates used with the Reyniers sampler. Air was drawn into the impingers at a rate of 1 cubic foot per minute.

A total of four air samplings was obtained. The first was taken for 5 minutes immediately after aerosolization of bacteria; disinfectant was then sprayed into the room, and the other air samplings of 10 minutes' duration were taken at approximately 10, 30, and 60 minutes after aerosolization. The samples were serially diluted and plated with Trypticase** soy agar medium. After incubation, bacterial colonies were counted and recorded as VP/ft.^{3}

RESULTS

Results showing the effect of three different concentrations of ozone on the airborne bacteria are given in Table 1. The reduction in the number of airborne microorganisms is expressed in mean percent reductions. These were computed from percent recovery values obtained from microbial concentrations recovered both with and without ozone exposure. Initial concentrations of airborne streptococci and staphylococci of about 175 VP/ft.^{3} were significantly reduced†† by 100 and 92 percent within 5

---

*Rodac Plates, Falcon Plastics, Los Angeles, Calif.
†Ozium, No. 1500, Woodlets Inc., Buffalo. N.Y.
‡Air-Fresh, Cetylite Industries, Long Island City, N.Y.
§Calculated to be 10 mg. total glycols per liter of air.
**Baltimore Biological Laboratories, Inc., Baltimore, Md.
††$p < 0.01$, by the Student t Test.

minutes after exposures to 1.0 p.p.m. ozone. At the TLV of 0.1 p.p.m. ozone and less, no significant reductions* were noted. The effect of ozone on B. subtilis spores was not significant* at any concentration.

Table 1.--The Effect of Ozone on Airborne Microbial Reductions

Bacteria	Amounts of Ozone (p.p.m.)	Mean Microbial Reductions (%)* Time (minutes) 5	15	30	50	Samples per Category (No.)
S. mitis	1.0	100†	100	100	100	3
	0.1	72	84	95	99	6
	0.05	56	66	88	96	3
	None	56	66	87	93	12
S. epidermidis	1.0	92†	96	100	100	3
	0.1	61	56	87	97	6
	0.05	51	45	82	95	5
	None	52	49	67	94	12
B. subtilis spores	1.0	64	63	84	92	3
	0.1	47	32	58	84	6
	0.05	47	22	46	74	4
	None	47	29	48	78	10

*Values are mean percent reductions computed from daily initial microbial recoveries of approximately 175 V.P./ft.^{3}. The less than 5-minute percent reductions were computed from initial 1-minute recoveries made without ozone; all others were computed from initial 5-minute recoveries.

†Significantly reduced ($p < .01$, by the Student t Test).

After exposure for 1 hour to 0.1 p.p.m. ozone, surface microorganisms were reduced as follows: S. mitis 73 percent, S. epidermidis 36 percent, and B. subtilis spores 20 percent. (Not shown in Table 1.)

*$p > 0.05$, by the Student t Test.

The results of the effects of glycol and water sprays on airborne microbial reductions are seen in Table 2. Values are mean percent

Table 2.--The Effect of Glycol Sprays on Airborne Microbial Reductions

Bacteria	Type of Spray	Mean Microbial Reductions (%)* Time (minutes) 10	30	60	Samples per Category (No.)
S. mitis	Ozium	46	96	100	4
	Air-Fresh	53	90	99	6
	Water (control)	42	88	98	5
	None (control)	45	88	98	7
S. epidermidis	Ozium	60	92	96	9
	Air-Fresh	28	87	96	8
	Water (control)	46	77	92	8
	None (control)	50	76	90	11
B. subtilis spores	Ozium	0	18	43	5
	Air-Fresh	0	48	50	6
	Water (control)	0	32	53	5
	None (control)	0	32	49	5

*Values are mean percent reductions computed from initial microbial recoveries obtained prior to spraying with disinfectant.

reductions computed from 60-minute microbial recoveries. The 60-minute percent reductions were computed from initial counts obtained immediately after aerolization of bacteria, and after aerosolization at the three recovery times. Although mean percent reductions in the number of the three bacteria steadily increased in most cases with successive recovery times, no significant differences* were noted in percent reductions obtained with Ozium or Air-Fresh, when compared with the controls.

DISCUSSION

Bacteria such as streptococci and staphylococci can cause many diseases in humans by the airborne route.[17-20] Although these bacteria are commonly

*$p > 0.05$, by the Student t Test.

found in the air of dental operating rooms,[21-23] no link between them and cross infection in DOR's has been definitely established. DOR's however, like other patient treatment areas, are usually managed in accordance with the sanitary approach. Because of the emphasis on sanitation, new procedures and devices are continually being introduced in an attempt to reduce microbial contamination in DOR's.

Ozone in concentrations of 1.0 p.p.m. was found, in our study, to effectively reduce the number of airborne streptococci and staphylococci. It would appear from these findings that the use of an ozone generator might be a practical solution to the problem of airborne contamination except for the overwhelming evidence that ozone at concentrations of 1.0 p.p.m. and above is toxic to man and animals upon prolonged inhalation.[10] This finding has resulted in reestablishment of the TLV for ozone at 0.1 p.p.m. In this study, however, ozone did not significantly reduce airborne contamination at 0.1 p.p.m. The question may be asked whether higher concentrations of ozone might be used when no one was in the room. This alternative is not feasible, because ozone has been shown to degrade materials like elastomers and textiles,[24] which are commonly found in DOR's. It was also observed in this study that ozone concentrations in the room could not be adequately controlled without constant manipulation of the ozone generator. This limitation could result in attainment of hazardous concentrations of ozone in DOR's during decontamination procedures.

A suggested use for ozone in the DOR may be in the decontamination of the warm water systems of dental units, which have been found to be heavily contaminated with microorganisms.[25] Further studies in this area are needed.

Glycol-type spray disinfectants have also been introduced to the dentist with vague claims for killing or reducing the number of airborne bacteria. It is known that certain concentrations of glycol vapor are toxic to many bacteria, but the effectiveness of commercial preparations of glycol in combination with other chemicals, applied as liquid sprays, is questionable. Our findings showed that microbial air reductions obtained with the glycol aerosols were no different from those obtained with water aerosols. Although the amount of glycol disinfectant sprayed into the room was in excess of the manufacturers' recommended dosages, it can only be speculated that the amounts were insufficient to produce the vapor concentrations needed for microbicidal activity.

It was concluded that ozone is effective against airborne bacteria but only at concentrations greater than the toxic level for humans, and also that commercially available glycol spray disinfectants used in this study are ineffective when used at recommended dosages.

SUMMARY

A dielectric type generator (OzoneAir) and two commercial glycol-type spray disinfectants (Ozium and Air-Fresh) were evaluated in a closed room for effectiveness in reducing the number of airborne bacteria. Ozone in concentrations of 0.05, 0.1 (the threshold limit value for humans), and 1.0 p.p.m., and the two commercial glycol aerosols, were tested in a 700-ft.^{3} capacity closed room for their effects on reductions in the number of airborne S. mitis, S. epidermidis, and B. subtilis spores. At ozone

concentrations of 1.0 p.p.m., more than 90 percent of the streptococci and staphylococci were removed from the air within 5 minutes. No airborne reductions were noted at the TLV (threshold limit value) concentration of ozone. The effect of the glycol aerosols on 60-minute reductions of airborne bacteria was no different from water aerosol controls.

ACKNOWLEDGMENTS

We acknowledge with thanks the advice of Robert A. Johnson, Industrial Hygienist, Navy Toxicology Unit, National Naval Medical Center, and the technical assistance of Howard D. Pinkston, Dental Technician Third Class, USN.

REFERENCES

1. Coblentz, W. W. Ultraviolet radiation and ozone as aerial disinfectants. Arch Phys Ther 13:709-711, 1942.
2. [illegible] W. J., and Van Den Ende, J. An investigation of the merits of ozone as an aerial disinfectant. J Hyg (Camb) 42:240-265, 1942.
3. Greenberg, L. Effect of germicidal aerosol treatment on the microbial flora of laboratory air. J Pharm Sci 55:740-742, 1966.
4. Puck, T. T.; Robertson, O. H.; and Lemon, H. M. Bactericidal action of propylene glycol on microorganisms suspended in the air II. J Exp Med 78:387-406, 1948.
5. Robertson, O. H.: Bigg, E.; Miller, B. F.; Baker, Z; and Puck, T. T. Sterilization of the air by certain glycols employed as aerosols. Science 93:213-214, 1941.
6. Robertson, O. H.; Bigg, E.; Puck, T. T.; and Miller B. F. The bactericidal action of propylene glycol vapor on microorganisms suspended in the air I. J Exp Med 75:593-610, 1942.
7. Lester, W., Jr.; Kaye, S.; Robertson, O. H. ; and Dunklin, E. W. Factors of importance in use of triethylene glycol vapor for aerial disinfection, Am J Public Health 40:813-820, 1950.
8. Cathcart, W. H.; Ryberg, R. E.; and Merz, A. I. The effect of ultra-violet radiation and ozone on bakery custard and cream fillings. Food Res 7:1-9, 1942.
9. American Conference of Governmental Industrial Hygienists. Threshold Limit Values for Substance in Workroom Air Adopted by ACGIH for 1972, p. 23.
10. Stokinger, H. E. Ozone toxicology. Arch Environ Health 10:719-731, 1965.
11. Robertson, O. H.; Loosle, C. G.; Puck, T. T.; Lemon, H. M.; Wise, H.; and Lester, W., Jr. Test for chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. J Pharmacol Exp Ther 91:52-76, 1947.
12. Hamburger, M., Jr.; Puck, T. T.; and Robertson, O. H. The effect of triethylene glycol vapor on air-borne beta hemolytic streptococci in hospital wards, #I. J Infect Dis 75:208-215, 1945.
13. Harris, T. N., and Stokes, J., Jr. Summary of a 3-year study of the clinical application of the disinfection of air by glycol vapors. Am J Med Sci 209:152-156, 1945.
14. Personnel of United States Naval Medical Research Unit No. 4. The use of triethylene glycol vapor for control of acute respiratory diseases in Navy recruits. II. Effect on acute respiratory diseases. Am J Hyg 55:215-229, 1952.
15. Shreve, W. B., and Wachtel, L. W. Effect of extraneous particulate matter on airborne bacteria. J Dent Res 49:589-592, 1970.
16. Bond, R. G.; Halbert, M. M.; Keenan, K. M.; Putnam, H. D.; Ruschmeyer, O. R.; and Vesley, D. (Excerpts from) Development of a Method for Microbial Sampling of Surfaces with Special Reference to Reliability. University Health Service and School of Public Health, University of Minnesota, Minneapolis, Minn. July 1963.

17. Langmuir, A. D. "Epidemiology of Airborne Infection" In W. McDermott, ed. Conference on Airborne Infection. Miami Beach, Fla., December 7-10, 1960. Baltimore. The Williams & Wilkins Co.
18. Miller, L. F. Acute Respiratory Infections in Naval Personnel. Proc First Int Symp Aerobiol. Berkeley, Calif., pp.3-23, 1968.
19. Williams, R. E. O. Spread of Airborne Bacteria Pathogenic for Man. 17th Symposium of the Society for General Microbiology. Airborne Microbes. Cambridge University Press. pp.268-285, 1967.
20. Hayden, J. L. A preliminary aerobic bacterial study of rebounded sprays from utilization of the air rotor handpiece. J Kans State Den Assoc 53:10-13, 1969.
21. Mazzarella, M. A., and Flynn, D. D. "Dental Aerosols," An Introduction to Experimental Aerobiology, New York: Wiley-Interscience, Chap. 18, 1969.
22. Pelleu, G. B., Jr.; Shreve, W. B.; and Wachtel, L. W. Reduction of microbial concentration in the air of dental operating rooms: I. High efficiency particulate air filters. J Dent Res 49:315-319, 1970.
23. Larato, D. C.; Ruskin, P. F.; and Martin, A. Effect of an ultrasonic scaler on bacterial counts in air. J Periodontal 38:550-554, 1967.
24. National Air Pollution Control Administration Publication No. AP-63, U.S. Department of Health, Education and Welfare. Air Quality Criteria for Photochemical Oxidants, "The Effect of Ozone on Materials," Chap 7, 1970.
25. Pelleu, G. B., Jr., and Wachtel, L. W. Dental equipment may spread microorganisms. J Environ Health 33:371-375, 1971.