

Ozone killing action against bacterial and fungal species; microbiological testing of a domestic ozone generator

A DYAS, BJ BOUGHTON, BC DAS

From the Departments of Microbiology and Haematology, Queen Elizabeth Hospital, Edgbaston, Birmingham

SUMMARY The action of ozone generated from a small domestic device was examined with a view to using it in clinical isolation units accommodating immunosuppressed patients. Over a six-hour period in an average size room the device did not generate sufficient ozone to suppress bacterial and fungal growth. A useful bactericidal action, against a variety of human pathogens was achieved with ozone concentrations between 0.3 to 0.9 ppm. Bactericidal ozone concentrations are close to the limit permitted for human exposure however and further experiments are indicated.

Ozone has well documented bactericidal properties which are of use in the decontamination of uninhabited "bioclean" areas.^{1,2} The purpose of the present study was to determine the effectiveness of an inexpensive domestic ozone generator in maintaining an ultra clean environment for immunosuppressed patients.

Material and methods

The Miles and Misra technique of bacterial enumeration³ was modified as follows. Peptone water suspensions were made from plate cultures of *E coli*, *Proteus* sp, *Pseudomonas aeruginosa*, *Serratia* sp, three strains of *Staphylococcus aureus*, *Candida albicans* and *Aspergillus fumigatus*. Subsequent ten-fold dilutions were made in sterile water. Two separate 20 µl drops of each dilution were then delivered on to each of two predried plates. Blood agar was used, except for *Proteus* sp where cysteine lactose electrolyte deficient (CLED) agar was used and for *Aspergillus fumigatus* where Sabouraud's agar was used. One plate was exposed to ozone for 4 h, the duplicate being placed in an ozone free environment. After 4 h all plates were incubated overnight at 37°C and the colonies counted using those dilutions which gave discrete colonies on both plates. All experiments were performed in duplicate.

Ozone was produced using a small "Airbracer"

produced by Coronair Ltd, Effingham, Surrey. The machine has a constant output of ozone and variable concentrations were produced by operating it in different test volumes. The main experiments used either a cupboard (0.087 m³) or a hospital room (66 m³). In the cupboard, the Airbracer was switched on at the beginning of the experiment and in the hospital room it was switched on two hours prior to commencing the experiments. Ozone concentrations were monitored using the Bendix 8002 analyser (Bendix Instruments, Lewisbury, West Virginia, USA).

Results

In the smaller space (0.87 × 10⁻²/m³), ozone concentrations varied between 0.3 and 0.9 ppm. These levels were reached within 10 min of switching on the machine. In the hospital room experiments, ozone was undetectable (despite an analyser sensitivity of 0.001 ppm).

The Table shows the CFUs surviving exposure to 0.3-0.9 ppm of ozone for 4 h. At this concentration ozone effectively inhibited the growth of all the bacterial species and *Aspergillus fumigatus*. *Candida albicans* was comparatively resistant however.

In additional experiments using plates exposed to ozone 0.9 ppm for 4 h prior to bacterial inoculation, it was possible to show that the effect of ozone on the agar was not bactericidal.

In the hospital room experiments at concentra-

Range of concentrations of seven bacterial and two fungal species (dispensed in 20 µl volumes) exposed to 0.3–0.9 ppm ozone for 4 h. The Table shows the number of cells surviving such treatment

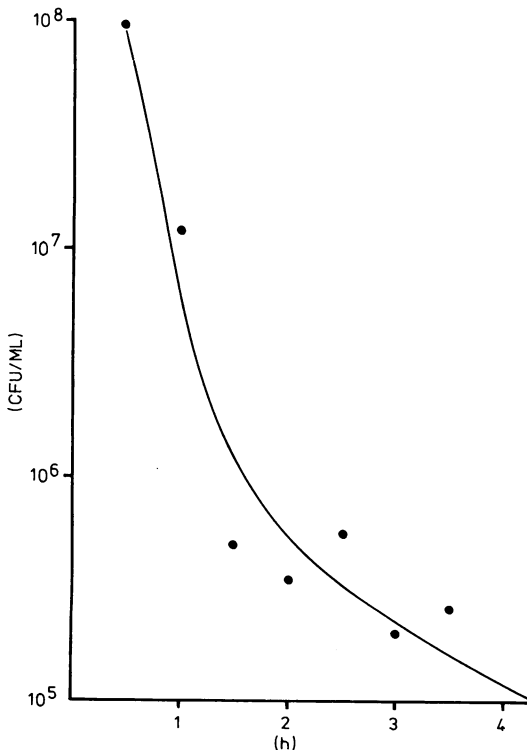
	Concentrations before ozone exposure (CFUs/ml)				
	10 ⁶	10 ⁵	10 ⁴	10 ³	10 ²
<i>Staph aureus</i>	5000	150	75	—	—
<i>Staph aureus</i> (penicillin-resistant)	*	450	75	—	—
<i>Staph aureus</i> (flucoxacillin-resistant)	*	*	25	—	—
<i>E coli</i>	—	*	*	0	—
<i>Ps aeruginosa</i>	—	—	*	125	50
<i>Serratia</i> sp	—	—	150	50	—
<i>Proteus</i>	—	50	25	25	—
<i>Aspergillus fumigatus</i>	—	—	800	—	—
<i>Candida albicans</i>	—	—	*	25	50

*Confluent colonies.

The results are a mean of two experiments.

tions of ozone below 0.001 ppm, no bactericidal effect was seen with any of the organisms tested.

Fig. 1 shows further experiments in which *Staph aureus* was exposed for 0–4 h to 0.9 ppm ozone. The bactericidal effect was most rapid within the first two hours of exposure but thereafter, the killing rate was slowed.



The number of surviving *Staph aureus* exposed to ozone, 0.3 to 0.9 ppm for 0–4 h.

Discussion

Our studies have demonstrated that when operating in a small space, the generator produced bactericidal concentrations of ozone (in the order of 1 ppm). However, its use in a hospital single room produced undetectable concentrations of ozone, and no bactericidal effect could be demonstrated. Previous workers have demonstrated that the minimum bactericidal concentration of ozone is 1 ppm,⁴ whilst other studies have suggested values between 20 ppm and 40 ppm.^{1,2} Broadwater and colleagues⁵ described bacterial killing by ozone as an “all or none” effect but Hamelin and Chung⁴ showed a progressive effect occurring over 1 ppm. In our studies, 1 ppm ozone was sufficient to reduce the numbers of most of the species tested by greater than 95%.

Hibben and Stotzkey⁶ examined the effect of ozone on fungal spore germination. Compared with bacteria, they found fungi to be less sensitive. *Aspergillus* spp. were found to be of intermediate sensitivity and required 1–1.5 ppm for six hours to inhibit germination. Our own experiments showed *Aspergillus* sp and *C albicans* to be less sensitive to ozone.

It is disconcerting however that in low ozone concentrations, mutations have been reported. Hamelin and Chung described the appearance of four mutant strains of *E coli* growing in 0.1 ppm ozone.⁴

Our work was carried out to examine the possible use of this small ozone generator to decontaminate rooms containing immunosuppressed patients. Ozone, at high concentration, has been used to disinfect rooms prior to patient occupation,² but these concentrations are toxic to man.^{7,8} Mortality rates are increased in exercising mice exposed to 0.3 ppm ozone for three hours and then challenging them with an aerosol of *Strep pyogenes*.⁹ In mice exposed

to 2.5 ppm for five hours and allowed to inhale *Staph aureus*, the rate of phagocytosis of pulmonary bacteria slowed compared with controls.¹⁰ In addition Peterson and colleagues¹¹ studied polymorph function in healthy male human volunteers exposed to 0.35–0.42 ppm ozone for 4 h. They found that, at 72 h after exposure, both bacterial killing and phagocytosis were moderately but significantly decreased.

We conclude that our domestic ozone generator did not produce bactericidal concentrations of ozone when operating in a hospital room. Higher and bactericidal ozone concentrations may have unacceptable toxic side effects to man but this, like the effect on airborne bacteria remains to be established.

We would like to thank Coronair Ltd for the loan of their Airbracer and Dr Alistair Kerr, Department of Chemistry, Birmingham University for the loan of the ozone analyser. Dr B Boughton is Senior Lecturer to the Leukaemia Research Fund.

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Requests for reprints to: Dr BJ Boughton, Department of Haematology, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, England.