Effect of Temperature on the Sterilization of Isopropyl Alcohol by Liquid Propylene Oxide

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Received for publication 1 April 1975

Liquid propylene oxide added to a solution of isopropyl alcohol and incubated at different temperatures markedly reduced the time required to sterilize the alcohol solution.

A previous report (6) described the sporicidal effect of adding liquid propylene oxide to a variety of nonsterile disinfectant solutions currently used in the medical field. One of these solutions, namely, isopropyl alcohol, is commonly used for preoperative skin disinfection in the form of impregnated surgical cotton swabs and in pharmaceutical lotions (2). Previous studies (6) with liquid propylene oxide were conducted at a temperature of 16 C. This report describes the sterilization of a solution of isopropyl alcohol using liquid propylene oxide at various temperatures above 16 C.

A spore suspension of Bacillus subtilis var. niger NCTC 10073 was prepared by a method similar to that of Beeby and Whitehouse (1). Solutions of 70% isopropyl alcohol (vol/vol) (B.D.H. Ltd., London) containing 10⁶ spores/ml were prepared. Propylene oxide at concentrations of 1% (wt/vol) and 5% (wt/vol) was added to the solutions. The solutions were stored in sterile glass ampoules at 16, 20, 25, and 30 C. Sporicidal tests were performed by sampling 1.0 ml of the solution from the ampoule into 50 ml of nutrient broth (Oxoid Ltd., London), followed by aerobic plate counts in nutrient agar (Oxoid Ltd., London). Two separate ampoules of each solution were used for each determination. Incubation of the nutrient agar plates was at 37 C for 48 h.

Similar determinations were performed by using the spore suspension and 70% isopropyl alcohol without the addition of propylene oxide.

The ability of the dilution in nutrient broth to neutralize the antibacterial properties of each solution was determined experimentally and found to be satisfactory.

Sporicidal tests (Table 1) showed that 5% propylene oxide was effective in sterilizing, after 9 to 10 days at 16 C, a solution of 70% isopropyl alcohol which had been contaminated with B. subtilis spores. However, 1% propylene oxide in 70% isopropyl alcohol at the same temperature showed little sporicidal effect (Table 1). These results were similar to results obtained previously (6), when it was shown that the sporicidal effectiveness of liquid propylene oxide was dependent on concentration. Sporicidal tests (Table 1) indicated that the effectiveness of liquid propylene oxide at concentrations of 5 and 1% were markedly enhanced at temperatures greater than 16 C. At 30 C, 5% propylene oxide was effective in sterilizing the isopropyl alcohol solution within 1 to 2 days, compared to a time of 9 to 10 days at 16 C. Similarly, 1% propylene oxide was effective in sterilizing the isopropyl alcohol solution at 30 C, but was ineffective at 16, 20, and 25 C. Reports in the literature (3, 4) on sterilization by gaseous propylene oxide indicate that its effectiveness as a sterilant is enhanced with increases in temperature. Our results indicate that this is also true for propylene oxide in the liquid form, and this is in agreement with the general principles of disinfection rates at increased temperatures (7).

Since the residual concentration of propylene oxide, particularly after its addition to food-stuffs, is stringently controlled by regulations (5), then by using liquid propylene oxide in conjunction with temperature the effective concentration of propylene oxide added to the material may be reduced. Therefore, it appears that addition of propylene oxide in the liquid form to isopropyl alcohol solutions at the most suitable temperatures may provide an effective and simple method for sterilization of such disinfectant solutions.

We wish to thank L. A. Percival for technical assistance.
### Table 1. Effect of temperature on the sterilizing properties of propylene oxide against B. subtilis spores

| Solution storage temp (°C) | Time of sampling (days) | Spore count/mL* | Test solution with PO (5%, wt/vol)* | Test solution with PO (1%, wt/vol)* | Control solution without PO* |
|----------------------------|-------------------------|-----------------|-------------------------------------|-------------------------------------|-----------------------------|
| 16                         | 1                       | $5.1 \times 10^3$ | $7.7 \times 10^3$                   | $9.3 \times 10^3$                   |                             |
|                            | 2                       | $1.3 \times 10^3$ | $6.7 \times 10^3$                   | $8.5 \times 10^3$                   |                             |
|                            | 3                       | $2.8 \times 10^4$ | $4.6 \times 10^3$                   | $5.0 \times 10^3$                   |                             |
|                            | 4                       | $1.5 \times 10^4$ | $4.5 \times 10^3$                   | $5.5 \times 10^3$                   |                             |
|                            | 5                       | $8.0 \times 10^3$ | ND†                                | $3.9 \times 10^3$                   |                             |
|                            | 6                       | $6.4 \times 10^3$ | ND                                  | $2.8 \times 10^3$                   |                             |
|                            | 7                       | $7.0 \times 10^3$ | $2.2 \times 10^3$                   | $5.0 \times 10^3$                   |                             |
|                            | 8                       | $6.4 \times 10^3$ | $1.5 \times 10^3$                   | $3.8 \times 10^3$                   |                             |
|                            | 9                       | $10^3$            | $8.1 \times 10^4$                  | $2.6 \times 10^5$                   |                             |
|                            | 10                      | $0$              | $8.9 \times 10^4$                  | $2.2 \times 10^5$                   |                             |
|                            | 11                      | $0$              | $8.5 \times 10^4$                  | $2.0 \times 10^5$                   |                             |
|                            | 12                      | $0$              | ND†                                | $1.7 \times 10^6$                   |                             |

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* Mean of two determinations. PO, Propylene oxide.

† Initial spore counts: test solution with 5% PO, $8.2 \times 10^8$; test solution with 1% PO, $10^6$; control, $1.1 \times 10^6$.

ND. Not determined.