J. Hyg., Camb. (1983), **91**, 287–292
Printed in Great Britain

The sterilizing capacity of propylene oxide and chlorhexidine diacetate solutions upon pre-injection swabs saturated with propan-2-ol

By R. C. WRIGHT*

Everett Medical Products Ltd, 2–10 *Commonside East*, *Mitcham*, *Surrey*

(*Received* 7 *February* 1983; *accepted* 23 *May* 1983)

SUMMARY

The sporicidal activity of two solutions which have been used in the production of 'sterile' pre-injection swabs has been investigated. Propylene oxide (3·4 % w/w) and chlorhexidine diacetate (0·6 % w/w), both made up in approximately 65 % w/w propan-2-ol, were artificially contaminated with spores of *Bacillus subtilis* var. *niger* and swabs were impregnated with these; surviving spores were enumerated at various temperatures and times of storage. Chlorhexidine diacetate had no sporicidal activity, whereas the activity of propylene oxide was dependent on temperature. The former should not be considered a sterilant and the latter can only be considered such if a controlled incubation period at an elevated temperature is employed.

INTRODUCTION

Sterility of medical products is an absolute condition which can never be proved; only the probability of sterility can be assessed. The manufacturers and distributors of medical products must therefore be able to demonstrate the probability of sterility of any product for which sterility is claimed. Such a demonstration normally involves evidence of (*a*) sufficient production control and (*b*) the efficiency of the final sterilization process employed. The former is provided by the achievement of consistently low pre-sterilization contamination levels of the product. The latter is provided by some form of challenge-testing: artificially contaminating the product with large numbers of a test organism known to be resistant to the relevant sterilization process. With adequate data it is then possible to prove the probability of, say, one article in 10^6 articles being contaminated with one viable organism after sterilization, a condition usually sufficient to allow a claim for sterility.

Production of pre-injection swabs involves cutting the swab material to size, inserting it into laminated-foil sachets, adding the desired solution and, finally, sealing the sachets. These processes lend themselves to mechanization and there is little direct human contact, reducing the risk of microbial contamination. However, the raw materials used for production are often already contaminated with low numbers of (predominantly spore-forming) organisms derived from air

* Present address: Analytical Laboratory, Njala University College, P.M.B., Freetown, Sierra Leone.

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press

and dust, mainly species of *Bacillus*. Whilst the swab solution is normally filter-sterilized immediately prior to use, the other raw materials are not sterilized. It is also possible that failure of the filters used for the solution could lead to the substantial contamination of some swabs.

Despite the possibility of contamination during production, some of the different pre-injection swabs available have apparently undergone no proven sterilization process; moreover, these may bear a claim to sterility. Liquid propylene oxide (PO), at 3–4% w/w, has usually been used as 'sterilant' and the capacity of this to sterilize solutions of propan-2-ol has been reported before (Hart & Brown, 1974; Hart & Ng, 1975). However, PO is normally used in other industries in the gaseous form (McBean & Johnson, 1959; Nury, Miller & Brekke, 1960; Bruch & Koesterer, 1961; Blanchard & Hanlin, 1973). Chlorhexidine diacetate, at 0·1–1% w/w, has also been used as an additive to pre-injection swabs impregnated with propan-2-ol, sometimes with and sometimes without PO. Some of these swabs may also bear a claim to sterility.

Sterility tests performed on finished swabs (before distribution), produced as described above, by one manufacturer, repeatedly produced small numbers of positive results (usually caused by spore-forming *Bacillus* spp.). It was therefore considered that the addition of liquid PO and/or chlorhexidine diacetate to pre-injection swabs did not represent a 'proven sterilization process' and required investigation.

MATERIALS AND METHODS

Test organism

Bacillus subtilis var. *niger* NCTC 10073 was cultured and spores harvested according to Beeby & Whitehouse (1965). A final spore suspension of approx. 10^7 spores ml^{-1} was prepared in deionized water. This organism is used for the routine monitoring of ethylene oxide sterilization and was that used by Hart & Brown (1974) and Hart & Ng (1975). Its resistance to PO was reported by Bruch & Koesterer (1961).

Test and control mixtures

Four mixtures were prepared, as follows. (All solutions were filtered through Oxoid 0·45 μm membrane filters before addition of the spore suspension.)

	g
1. Propan-2-ol	987
PO	51
Spore suspension	462
2. Propan-2-ol	953
Chlorhexidine diacetate	9
Spore suspension	538
3. Propan-2-ol	990
Spore suspension	510
4. Quarter-strength Ringer's solution	1000
Spore suspension	500

Mixtures 1 and 2 are equivalent to ones which have been used in the production of pre-injection swabs (using deionized water in place of the spore suspension). Mixtures 3 and 4 serve as controls.

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press

Trials

One ml of mixture 1 was introduced into each of 100 sterilized laminated-foil sachets containing standard, dry, pre-injection swabs and the sachets were heat-sealed. Similarly, 1 ml of each of mixtures 2–4 was introduced into each of 100 sachets + swabs. Ten swabs from each batch of 100 sealed sachets were then transferred to 90 ml diluent (quarter-strength Ringer's solution), thoroughly shaken and serial decimal dilutions of the diluent made; appropriate dilutions were filtered through Oxoid 0·45 μm membrane filters and filters incubated for 3 days at 30 °C on yeast extract agar (Oxoid CM 19). The counts of spores per swab at zero time were calculated from results.

The remaining 90 sachets from each batch were divided, 45 being placed in a refrigerator (mean temperature = 2 °C) and 45 in an incubator at 28 °C. After 1, 2, 3, 4, 7, 10 and 14 days storage, spore counts were made by transferring five swabs from each sub-batch of 45 to 45 ml diluent and then proceeding as described above for the determination of counts at zero time. If, at any time of storage, sterility of swabs seemed to have been achieved, sterility tests were performed by transferring individual swabs directly to 100 ml nutrient broth (Oxoid CM 67), followed by incubation at 30 °C for 3 days.

After the results of the above trials were obtained, the influence of temperature on survival of spores of the test organism in the presence of PO was investigated in more detail by introducing 1 ml of mixture 1 into each of 200 sterilized sachets containing dry swabs, sealing the sachets and incubating 50 of these at each of the following temperatures: 15, 20, 25 and 37 °C. At relevant times of storage for up to 21 days, five swabs were spore-counted as described above (or sterility-tested).

A sample of mixture 1 was incubated in a closed bottle at refrigerator temperature (mean = 4 °C) for 14 days; another sample of this mixture was incubated at 30 °C for 14 days. After this period, the concentration of PO remaining was estimated by reaction of 5·0 ml of the mixture with a standard volume of approx. 0·5 M-HCl (made up in propan-2-ol), followed by titration with 0·1 M-NaOH to the phenolphthalein end-point. (A blank of 5·0 ml 65% w/w propan-2-ol was similarly run.)

The possibility of seepage of PO and/or propan-2-ol from sealed sachets at elevated temperatures was investigated by weighing 20 sachets, containing swabs impregnated with mixture 1, incubating these at 37 °C for 21 days, and then re-weighing them.

RESULTS

The sporicidal activity of PO was dependent on the temperature of storage (Table 1); activity was nil at 2 °C, whilst approx. 10^6 spores were killed in a period of 7–10 days at 28 °C. Chlorhexidine diacetate + propan-2-ol and propan-2-ol alone displayed no sporicidal activity. A slight increase in numbers of the test organism occurred in the control swabs containing quarter-strength Ringer's solution and incubated at 28 °C, probably due to the presence of some nutrient material(s) in the swab or laminated-foil sachet.

The relationship between storage temperature (between 15 and 37 °C) and the sporicidal activity of PO (Fig. 1) can be expressed mathematically as $y = e^{4\cdot26-0\cdot1x}$,

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press

Table 1. *Counts* of* Bacillus subtilis *var.* niger *from contaminated swabs impregnated with different mixtures and stored at 2 or 28 °C for various times*

Time of storage (days)	Swabs impregnated with: Mixture 1		Mixture 2		Mixture 3		Mixture 4	
	2 °C	28 °C	2 °C	28 °C	2 °C	28 °C	2 °C	28 °C
0	6·04		6·32		6·04		6·26	
1	6·18	5·54	6·40	6·46	6·26	6·18	6·89	6·56
2	6·04	5·32	6·20	6·20	6·20	6·23	6·23	6·90
3	6·11	4·43	6·57	6·46	6·28	6·30	6·26	7·04
4	6·18	1·85	6·30	6·18	6·36	6·18	6·45	7·00
7	6·20	0·30	6·30	6·26	6·28	6·30	6·78	6·88
10	6·18	$< \bar{1}\cdot30$†	6·36	6·26	6·28	6·20	6·48	7·18
14	6·08	$< \bar{2}\cdot82$‡	6·18	6·38	6·28	6·18	6·59	7·15

* Recorded as $\log_{10}$ mean counts per swab ($n = 5$).
† Five swabs were individually sterility-tested; all tests were negative.
‡ Fifteen swabs were individually sterility-tested; all tests were negative.

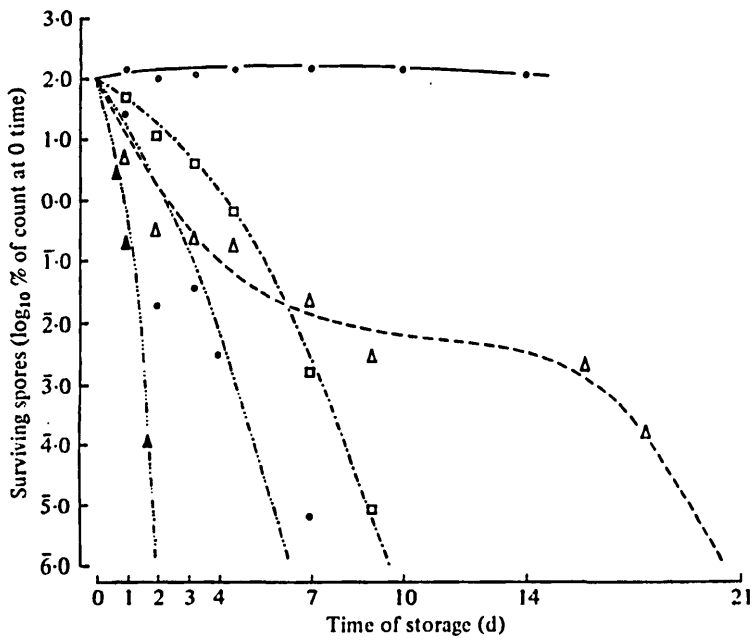

Fig. 1. Influence of storage at different temperatures on the sporicidal activity of propylene oxide. O——O, 2 °C (data taken from Table 1); △-----△, 15 °C; □-·-·-·□, 20 °C; ●-·-·-·-·-●, 25 °C; ▲-·-·-·-·-▲, 37 °C.

where y = the time (in days) required to produce a reduction by a factor of 10^6 in the number of spores originally contaminating swabs, at a temperature of x °C. The correlation coefficient for this relationship, using the data from Fig. 1, is 0·989, which is significant at $P < 0{\cdot}05$.

The concentration of PO remaining in solution after 14 days storage at 4 °C was 2·8% w/w, and at 30 °C was 2·4% w/w.

The mean loss of weight from filled sachets after incubation for 21 days at 37 °C was 0·7%.

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press

DISCUSSION

Chlorhexidine diacetate cannot be considered a sterilant for pre-injection swabs. Furthermore, in the short time interval between skin cleansing with a chlorhexidine diacetate-impregnated swab and injection, it might be difficult to show a significant bactericidal effect upon the normal skin flora. Chlorhexidine diacetate could therefore be considered a redundant additive to pre-injection swabs.

Liquid PO also cannot be considered a sterilant for pre-injection swabs, unless these are submitted to a post-production incubation period at an elevated temperature. This temperature should be > 15 °C, as a significant tailing-off of the death curve for the test organism used herein occurs at this temperature (Fig. 1), a period of approx. 18 days being required to ensure a reduction by a factor of 10^6 in the original number of contaminating spores. This tailing-off was not observed by Hart & Ng (1975), who reported a kill of approx. 10^6 spores of the same test organism in 9–10 days at 16 °C. Hart & Ng were, however, using test solutions rather than test swabs, and a slightly higher concentration of PO (approx. 4 % w/w). The temperature of incubation should ideally be above the boiling-point of PO (approx. 34 °C), e.g. 37 °C. (Reduction of spores by a factor of 10^6 occurred in 1·8 days at this temperature.) However, using the formula $y = e^{4\cdot26-0\cdot1x}$, and with sufficient data on the normal pre-sterilization contamination levels of production swabs, the time of storage required at any particular temperature between 15 and 37 °C which would be necessary to produce a high probability of sterility can be calculated. It should be noted that this formula only applies to the use of PO at a concentration of 3·4 % w/w and that alteration of this concentration would alter the relationship between temperature and sporicidal activity (Hart & Brown, 1974).

Post-production incubation of swabs at elevated temperatures (up to 37 °C) for short periods (up to 21 days) would not significantly reduce the amount of mixture with which the swabs were impregnated. The decrease of PO content observed in solution over a period of 14 days could not entirely be accounted for by a conversion of liquid PO to gaseous PO and was probably due also to a conversion of some of the PO to a glycol.

It can be argued that pre-injection swabs need not necessarily be sterile as they are used only for topical application on (contaminated) skin surfaces. In this case, PO should also be considered a redundant additive and no claim for sterility made.

I would like to thank Dr A. Dadd for a culture of the test organism, Miss M. Lewis and T. Baker for preparing the mixtures and for the volumetric assay of residual PO, and Everett Medical Products Ltd. (in particular Mr C. G. Grenshaw) for providing the facilities and encouragement to perform this study.

REFERENCES

Beeby, M. & Whitehouse, C. E. (1965). A bacterial spore test piece for the control of ethylene oxide sterilisation. *Journal of Applied Bacteriology* **28**, 349–360.

Blanchard, R. O. & Hanlin, R. T. (1973). Effect of propylene oxide treatment on the microflora of pecans. *Applied Microbiology* **26**, 768–772.

Bruch, C. W. & Koesterer, M. G. (1961). The microbicidal activity of gaseous propylene oxide and its application to powdered or flaked foods. *Journal of Food Science* **26**, 428–435.

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press

HART, A. & BROWN, M. W. (1974). Propylene oxide as a sterilizing agent. *Applied Microbiology* **28**, 1069–1070.

HART, A. & NG, S. N. (1975). Effect of temperature on the sterilisation of isopropyl alcohol by liquid propylene oxide. *Applied Microbiology* **30**, 483–484.

MCBEAN, D. & JOHNSON, A. A. (1959). Control of spoilage in high-moisture prunes. *Food Preservation Quarterly* **17**, 48–51.

NURY, F. S., MILLER, M. W. & BREKKE, J. E. (1960). Preservative effect of some antimicrobial agents in high-moisture dried fruits. *Food Technology* **14**, 113–115.

https://doi.org/10.1017/S0022172400060307 Published online by Cambridge University Press