AN INDICATOR CONTROL DEVICE FOR ETHYLENE OXIDE STERILISATION

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Received May 22, 1959

The use of physical sterilisation controls instead of bacteriological controls for heat sterilisation processes is cited. Due to sorption of the gas and penetrability difficulties added need for such controls exists in ethylene oxide sterilisations. Some data on sorption of ethylene oxide is presented. The construction of a simple disposable device, to indicate the attainment of lethal conditions in ethylene oxide sterilisations is described. Data are presented to compare the control device with bacteriological controls, under different conditions of time, temperature, and gas concentration.

The classic method of testing the effectiveness of any microbial sterilisation is to include in the sterilisation a suitable preparation of resistant organisms. Subsequently the preparation is cultured to ascertain whether the organisms were killed. Such a process is time-consuming, some days must elapse before even a reasonably certain answer can be given. It also requires specialised laboratory staff. In addition there are considerable difficulties in obtaining suitable resistant organisms, and in maintaining their resistance.

In the field of heat sterilisation many attempts have been made to produce devices which are suitable routine controls on sterilisers and which will give an immediate indication on completion of the process that it was effective. These range from various melt tubes and colour change papers, which indicate merely that a certain temperature was reached or exceeded momentarily, to more suitable devices which do indicate whether a certain time at temperature was exceeded or not.

This paper describes the extension of the "physical control" to gas treatments in general, and to ethylene oxide sterilisations in particular.

Ethylene oxide has been used for more than 20 years as an insecticidal fumigant and as a sterilising agent against micro-organisms. Its initial use was covered by various patents[1–5], but considerable disagreement as to the mode of usage was evident, in that some specified that moisture must be present whilst others that it should be absent.

It was not until the work of Phillips and Kaye[6–8] in 1949, that it had very much application as a sterilising agent against micro-organisms. Subsequently papers by Velu and others[9] 1942, Roberts and others[10] 1943, Royce and Sykes[11] 1955, Rausher and others[12] 1957, and Grundy and others[13] 1957 all described its use and application to many sterilisation purposes but again there was considerable variation in the gas concentrations, time of exposure and temperatures specified.

Whilst some of this confusion may be due to the "partly protected" state of organisms in various substances treated, and to variations in

humidity, much is probably due to sorption of the gas by the materials being treated.

Although in its application to insecticidal fumigation[14] and in other connections[15] the sorption of ethylene oxide was well understood, there are few references in the field of sterilisation to sorption effects. Since these questions of sorption were largely the stimulus prompting the production of the sterilisation control described later, we have included a selection of sorption data which illustrates the wide differences between different materials and products. In presenting this we have attempted to relate it quantitatively to practical sterilisation conditions (Table I).

In any practical sterilisation, the amount of ethylene oxide absorbed depends upon the gas concentration and the amount of sorptive materials

TABLE I

SORPTION OF ETHYLENE OXIDE BY VARIOUS MATERIALS

Material	Amount of ethylene oxide sorbed after 18 hr. contact with 10 per cent v/v ethylene oxide in air at room temp. (20° C. approx.) mg./g.
Polythene	2
P.V.C.	19·2
Bakelite	Nil
Brown paper	6·1
Cardboard	10·4
Packing case wood	18·4
Cotton wool (absorbent)	3·5
Cotton wool (non-absorbent)	4·1
Red rubber closures	5·5
White rubber closures	7·4
Black (neoprene) rubber closures	15·2
Starch glove powder	10·5
Kaolin	2·5
French chalk	0·5
Sulphanilamide	0·8
Procaine penicillin	0·2
Water	25–30

present in the steriliser. Given sufficient time a state of equilibrium between the ethylene oxide concentration in the atmosphere of the steriliser and in the materials being treated will eventually be reached. The time required to reach this point depends on the nature of the absorbing material, its thickness, physical state, surface area, etc., and the temperature.

The death of an organism depends amongst other things on the gas concentration surrounding it, temperature, and time. Thus, if gas is sorbed by material in the steriliser, the concentration available to kill organisms is lowered and a consequently longer time will be required.

EXPERIMENTAL

When micro-organisms are exposed to lethal gases, the time required to kill the organisms at any given temperature varies inversely with the gas concentration, provided the organisms are not "protected" in some way from the gas, and provided that the conditions of relative humidity are appropriate. This lethal time-concentration product is roughly constant, over a wide range of gas concentrations; it varies inversely with temperature, a smaller one being lethal at higher temperatures, and a greater one being required at lower temperatures.

This has a close parallel in the diffusion of a gas through a permeable membrane. Here also the time required for a given quantity of gas to diffuse through a given area is inversely proportional to the gas concentration. Similarly at higher temperatures the diffusion rate is increased and at lower temperatures is reduced.

If therefore a quantity of a suitable absorbent for the gas containing a suitable indicator is enclosed in a gas-permeable envelope or sachet of controlled dimensions, then by variation of the quantity or strength of absorbent, or the dimensions of the sachet, it is possible to make an "artificial organism" which will change colour after the absorption of a certain quantity of gas. If it can also be arranged that this change takes place after the absorption of a lethal time-concentration product of the gas, a control is produced, which when included in a gas sterilisation, will indicate whether lethal conditions were attained at that place in the steriliser. Such indication would of course be apparent immediately on

TABLE II

TIME REQUIRED TO KILL SOIL DUST SPORES OR TO CHANGE THE SACHET CONTROL AT CONSTANT TEMP. (20° C.) IN VARYING CONCENTRATIONS OF ETHYLENE OXIDE

Ethylene oxide concentration per cent v/v in air or nitrogen	Time in hours required to	
	Sterilise soil dust containing 10^6 spores/g.	Change colour of sachet control, yellow to purple
100	<4	2
50	4–7	4
25	7–8	8
10	16	19
5	24	45
2·5	48	95

completion of the sterilisation and would require no further laboratory work. For ethylene oxide a suitable absorbent is a saturated aqueous solution of magnesium chloride containing hydrochloric acid[16]. This solution quantatively absorbs the gas to form ethylene chlorhydrin, the reaction of the solution becoming more alkaline.

Ethylene oxide is frequently employed in gas sterilisation processes mixed with large quantities of carbon dioxide to render the mixture non-explosive[17]. It is therefore necessary to use an indicator that is insensitive to carbon dioxide. There are many such indicators, we have chosen to use bromophenol blue.

Most plastics are readily penetrable by ethylene oxide and the envelope can be made of polythene, P.V.C. or nylon film, no doubt others could also be used. We have chosen for small scale work to use polythene. It is possible to make up envelopes of any dimensions from sheet material, but since "lay-flat" tubing in a great variety of widths and thicknesses is readily available it is more convenient to use this.

Since considerable divergence of view exists on the question of what constitutes a lethal time-concentration product for ethylene oxide, we have chosen to make these devices on the basis of our own work on ethylene oxide sterilisation. We have found over a period of years that a 10 per cent v/v gas concentration (200 mg./l.) for a time period of 16 to

18 hours at ambient temperatures is a convenient practical way of employing ethylene oxide. This process is based on the sterilisation of soil dust spores, which are admittedly difficult to sterilise. The devices can be manufactured to yield a positive result at any other time-concentration product if desired. The technical details of the device produced to control the practical sterilisation process outlined are included in the Appendix 1.

Tests were made with the devices in varying concentrations of ethylene oxide at a constant temperature (20°). Bacteriological soil dust spore preparations were also exposed to similar gas concentrations at the same temperature. These tests are summarised in Table II. A further series of tests was made using a constant concentration of ethylene oxide, at

TABLE III

TIME REQUIRED TO KILL SOIL DUST SPORES OR TO CHANGE THE SACHET CONTROL, IN A CONSTANT CONCENTRATION OF ETHYLENE OXIDE (10 PER CENT V/V), AT VARYING TEMPERATURES

	Time in hours required to	
Temperature ° C.	Sterilise soil dust containing 10^6 spores/g.	Change colour of sachet control, yellow to purple
10	32	32
20	16	19
30	7	9
40	5	6·5
50	4	4·5
60	3	3

different temperatures. Summarised results of these tests on soil dust spores and the sachet controls are given in Table III. Various tests with the devices have shown that they are sensitive to a concentration time product difference of approximately 10 mg. hr./l. This represents a difference of less than 0·5 per cent in the time-concentration product used in the sterilisation process quoted. Other slight variations occur in use, due to diffusion and mixing rates inside the sachet, and to slight dimensional differences in the sealed sachets. The sum of all such differences however does not introduce error greater than $\pm$ 2·5 per cent. This is considerably less than is tolerable for a steriliser control, and is certainly much smaller than the errors inherent in bacteriological controls.

DISCUSSION

It is evident that irrespective of wide differences in gas concentration, time of exposure and temperature, change of colour of the sachet control indicates that a lethal combination of these variables to micro-organisms existed at that point in a steriliser.

Due to sorption of the gas almost any ethylene oxide treatment must be a treatment with a varying gas concentration, and a control of the type described provides a means of integrating the total effect of such a treatment which would be difficult to achieve by other means. This is particularly so when one considers the different rates of diffusion and penetration of the gas into different types of materials and packages,

producing many differing concentrations, all changing at different rates, at various points in the materials being treated, in a particular sterilisation.

Whilst "blunderbuss" methods of greatly increased concentrations could no doubt resolve some of these difficulties, such methods are frequently self-defeating in that the increased concentrations cause increased sorption to occur. Additionally, deleterious change in the materials being treated is made more likely, and of course the cost of treatment is increased. A means of measuring the actual effect of the process at any point with a fair degree of accuracy can be a very valuable tool, in designing or evaluating sterilisation processes.

The principle can be applied to many other gases and to gas treatments other than sterilisation treatments. In the sterilisation field we have produced similar devices for formaldehyde treatments, and in the related field of insecticidal fumigation devices to control methyl bromide as well as ethylene oxide have been made. The applications to insecticidal fumigations will form the subject of another paper, which will be published elsewhere. Patent applications have been filed, and various types of these controls are now available.

Appendix

Specification for Control Sachet suitable for 10 *per cent v/v Ethylene Oxide Sterilisations at* 20° *for* 16 *to* 18 *hours*

Polythene "lay-flat" tubing of 0·005 in. thickness and 1 in. width is used.

This is cut into lengths of approx. $2\frac{1}{4}$ in. so that when both cut edges are sealed the finished sachet has a length of 2 in. One cut edge is then heat sealed. 5 ml. of the absorbent solution is introduced into the envelope.

The absorbent solution is saturated aqueous magnesium chloride solution containing 0·004 per cent bromophenol blue, acidified with hydrochloric acid so that 5 ml. of the solution is equivalent to 3 ml. 0·1 N HCl using bromophenol blue as indicator.

The other edge is heat sealed.

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After Mr. Royce presented the paper there was a Discussion.