CONTAMINATION CONTROL BY USE OF ETHYLENE OXIDE

TECHNOLOGY SUMMARY

(Task 12)

Contract NASw-2062

National Aeronautics and Space Administration
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by

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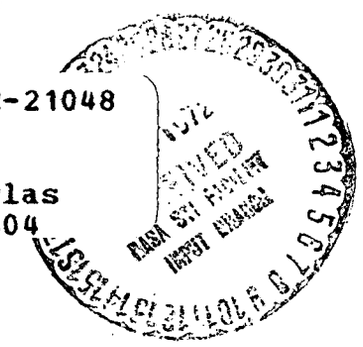

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FOREWORD

This document is a state-of-the-art summary prepared by Exotech Systems Inc. in partial fulfillment of contract NASW-2062, under the cognizance of the NASA Planetary Quarantine office.

The initial purpose of the report had been to summarize the technological advances in the application of ethylene oxide, which had been developed under the NASA Planetary Quarantine program. After a review of a preliminary draft, the scope of this paper was expanded to include the technological advances developed for non-space applications.

This state-of-the-art report should, therefore, be useful in evaluating the potential for new and further uses of ethylene oxide as a decontaminant for Planetary Quarantine related applications.

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INTRODUCTION

The requirements for Planetary Quarantine have prompted a review of spacecraft decontaminating agents to evaluate their effectiveness, and the feasibility of their use.

Ethylene oxide (ETO) is a highly penetrating and effective decontaminating gas, convenient to use, versatile, and effective at room temperature. It can kill all forms of microorganisms, including resistant soil varieties. The level of its effectiveness depends on such environmental variables as temperature, relative humidity, gas concentration, and duration of exposure. Other factors that affect the lethality are the age, and physical state, of the microorganisms.

While the probability of complete sterility through the use of ethylene oxide is less than that obtainable by the use of dry heat, the experimental data reviewed showed that at least a three order of magnitude reduction of the microorganism population could be achieved without difficulty.

The incompatibility of the ethylene oxide decontamination process with many materials that may be used on spacecraft is the greatest deterrent to its acceptability in spacecraft contamination control. In the decontamination process, the ethylene oxide and any water vapor that may be present can alter both chemical and physical properties of many metals and plastics. Prior to any use on spacecraft, therefore, care must be exercised in assuring the compatibility of all exposed materials.

I APPLICATIONS AND LIMITATIONS

Ethylene oxide has been known as an effective insecticidal fumigant since the 1920's. Its bacteriological activity was noted shortly thereafter. The main commercial application of ethylene oxide for sterilizing purposes appears to have been the treatment of spices which were often a source of food spoilage. It has also been used in medical and surgical practices, and in the pharmaceutical industries, to sterilize bedding, bandages, various types of medical instruments, and certain medical and biological preparations, including pinicilin and culture growth media.

Ethylene oxide has now been suggested as a possible surface decontaminent to reduce microbial populations on spacecraft (refs. 1 and 2). To date, it has not been used on any planetary flight project. Several factors indicate that great care must be taken to avoid contact between ethylene oxide and certain materials that may be used in spacecraft.

The most immediate hazards of ethylene oxide exposure are:

(a) Corrosion, caused by humidity, or condensed water, combined with a somewhat elevated temperature;

(b) Polymerization reactions, caused by catalytic properties of some materials;

(c) Chemical reaction with some of the materials used within the chambers (refs. 3, 4, and 5);

(d) Parameter drift of some devices (refs. 6 and 7).

Compatibility tests have been conducted on many materials used in spacecraft. In these tests, corrosion of metals, particularly of magnesium, was encountered. This was traced to a softening of conformal coating, with subsequent penetration of moisture which caused the corrosion.

Polymerization reactions are catalyzed by bases and organic salts. Resulting compounds are of low volatility, and may degrade electrical contacts and thermal control surfaces. Silver and copper can catalyze polymerization of ethylene oxide if moisture is present, and they can cause explosive reactions if acetylene contaminants are present in the ethylene oxide.

The compatibility tests included tests on adhesives, coatings, encapsulating compounds, elastomers, reinforced and unreinforced plastics, films and tapes (ref. 4). The tests consisted of exposing the materials to two environments: (a) heat of 150°C for three intervals of 40 hours each, and (b) a combination of 12 percent ethylene oxide and 88 percent Freon 12 for two periods of 24 hours.

About 20 to 25 percent of the materials evaluated were considered unacceptable after the heat sterilization. Only two materials (out of approximately 160) were appreciably degraded by ethylene oxide. Adverse long term effects, however, have been associated with exposure to ethylene oxide, indicating that it had been absorbed and retained in components (ref. 5). Liquid ethylene oxide is also a solvent

of such acrylic plastics as lucite and plexiglass, and will react with plasticizers employed in plastics for binding purposes.

Limited testing was also performed at Boeing (refs. 6 and 7) to establish the magnitude of the potential problems arising from ethylene oxide exposure. To accelerate short term results, parts were tested out of their normal-use environment, i.e., hermetic seals were breached and no protective conformal coating was used. Approximately 250 piece parts, of about 80 different types, were examined. The assortment of parts types included silicon transistors (both npn and pnp small signal and power devices), germanium semiconductors, various types of resistors and capacitors, typical integrated circuitry, and selected relays. All parts were of high reliability quality. The general test technique was to operate the devices in a normal mode, with power supplied for a full 24 hours at 40°C, in an ethylene oxide-Freon-water vapor decontamination gas.

Some difficulties were uncovered. The moisture associated with the process affected parameters that included transistor characteristics, loss of dielectric strength, minor drifts in capacitors, and similar difficulties. In every case, however, when the parameter drift was encountered, the defective device was repaired by high temperature bakeout. On test, drift was recreated by exposure to nitrogen and water vapor. The paints and labels on some relays were softened by the ethylene oxide exposure. Although the softening

was also reversible, sterilizable parts should not employ paints or varnishes that are soluble in ethylene oxide because of the potential long term degradation previously described.

II CHEMICAL AND PHYSICAL PROPERTIES

Ethylene oxide is highly penetrating and effective at room temperature (ref. 8). It is a colorless gas, liquifies readily at 10.8°C, and freezes at -111.3°C (ref. 9). The liquid is miscible with water as well as with other common organic solvents. It is the simplest cyclic ether or epoxy compound.

As little as 3.6 percent of ethylene oxide vapor in air is explosive and will support combustion (ref. 10). Therefore, other gases, such as carbon dioxide or the fluorinated hydrocarbons, which are biologically inert, are added to make a nonflammable mixture. When mixed with 90 percent carbon dioxide, or about the same percent of various fluorinated hydrocarbons, the resulting mixture can in turn be mixed with air in all proportions without passing through the flammable range (ref. 11).

The use of ethylene oxide may be severely limited because of polymerization. Polymerization can occur when ethylene oxide gas is in contact with moisture and catalysts such as acids, anhydrous chlorides of iron, tin, or aluminum, pure iron and aluminum oxides, and alkali metal hydroxides. The rate varies directly with increased temperature, pressure, and with the quantity of moisture (ref. 9). Its accumulation can cause severe damage to unprotected materials.

Polymerized ethylene oxide is found in varied forms including a white powder that occurs where water condensation accumulates, and a yellow oily liquid which can form a gummy solid.

III GERMICIDAL ACTIVITY

Ethylene oxide is one of the most widely used of the decontaminating gases. It kills all forms of microorganisms including resistant soil microorganisms (ref. 12). The mechanism by which it does so has been linked to its chemical activity as an alkylating agent (refs. 13 and 14). According to the alkylation theory, ethylene oxide replaces labile hydrogen atoms with hydroxy ethyl ($-CH_2CH_2OH$) groups, thus blocking many reactive sites needed in essential metabolic reactions.

A. INDEPENDENT VARIABLES AFFECTING EFFICIENCY

The relative resistance of microorganisms to ethylene oxide is a function of certain envrionmental variables such as sterilant concentration, exposure time, temperature, and moisture concentration. Unless these factors have appropriate values or configurations, significant decontamination will not take place, in spite of the presence of an adequate concentration of ethylene oxide (ref. 15). The following sections describe the significance of these factors. Also includes are considerations of the microorganism age, or level of development, and the organism population.

1. Sterilant Concentration

The decimal reduction time (D-value) is defined as the time required to reduce a given population of microorganisms in order of magnitude.

This value is generally reduced as the ethylene oxide concentration increases (refs. 16 and 17). The effect is demonstrated in Table I for various values of relative humidity. Spores of B. subtilis var. niger, were exposed on both hygroscopic and nonhygroscopic surfaces. The most marked effect was noted when the concentration was increased from 200 to 400 mg/liter. Considering all data at these two concentrations, irrespective of carrier materials and relative humidity, there was an average decrease in the D-values of 2.57 minutes. Note that this trend continues but diminishes considerably above 400 mg/liter. This indicates a leveling off of the effect, i.e., above a given concentration no significant increase in germicidal activity results from an increase in concentration.

2. Exposure Time

The death rates of several sporeforming and nonsporeforming microorganisms, including radiation-resistant cocci, were determined by exposing them to a mixture of ethylene oxide and dichlorodifluoromethane (500 mg/liter of ethylene oxide, at 30 to 50 percent relative humidity, and at 54.4°C). (See ref. 18.) Spore survivor curves were obtained from tests of inoculated and exposed hygroscopic and nonhygroscopic carriers. Table II shows the decimal reduction times derived from the curves. These results illustrate the relative variations in resistance among microorganisms when exposed to ethylene oxide, and disclose differences when organisms are dried on hygroscopic and nonhygroscopic surfaces.

TABLE I

DECIMAL REDUCTION VALUES OF B. subtilis var. niger SPORES[a] EXPOSED TO VARIOUS CONCENTRATIONS OF ETHYLENE OXIDE AT VARIOUS HUMIDITIES AT 54.4°C (From ref. 16)

Amt. of ethylene oxide(mg/liter)	15%		30%		50%		60%		90%	
	NHS[b]	HS[c]	NHS	HS	NHS	HS	NHS	HS	NHS	HS
200	5.75	6.75	7.50	7.50	6.25	5.00	7.00	7.50	7.25	5.50
400	4.00	3.50	3.75	3.75	3.75	3.50	3.00	3.75	4.75	4.50
600	4.25	2.75	3.75	3.25	3.75	2.75	3.50	3.00	3.75	3.00
800	2.75	2.50	3.25	2.25	3.25	3.50	4.00	2.25	2.50	2.25
1,000	2.50	1.75	2.25	2.00	2.00	2.50	1.50	1.50	2.25	1.50
1,200	1.25	1.75	1.50	1.75	1.25	1.50	1.50	1.75	1.75	1.50

a. Expressed in minutes as D values

b. Nonhygroscopic surface

c. Hygroscopic surface

TABLE II

DECIMAL REDUCTION VALUES OF VARIOUS SPOREFORMING AND NONSPOREFORMING ORGANISMS EXPOSED TO ETHYLENE OXIDE[a] (From Ref. 18)

Organism	NHS[b]	HS[c]
	min	min
Bacillus subtilis var. niger (Ft. Detrick)	6.66	4.30
Clostridium sporogenes (ATCC. 3584)	3.67	3.30
C. sporogenes (ATCC 7955)	3.25	2.80
B. stearothermophilus (ATCC 7953)	2.63	2.63
B. pumilus (ATCC 7061)	2.81	2.21
Micrococcus radiodurens	3.00	2.25
M. phlei (ATCC 11728)	2.40	1.40
Streptococcus faecalis (ATCC 349)	3.04	
S. faecalis (Ethicon F_6)	3.75	
S. faecalis (Ethicon A_21)	3.13	
S. faecalis (Ethicon 0_{12})	2.00	

a. Values are expressed as D values at 54.4°C, a concentration of ethylene oxide of 500 mg/liter, and 30 to 50 percent relative humidity.

b. Nonhygroscopic surface

c. Hygroscopic surface

3. Temperature

Within certain limits, higher temperatures enhance the germicidal activity of ethylene oxide by increasing penetration, and thus affording a reduction in exposure time. Each 10°C temperature increase, within the range of 5°C to 37°C, approximately doubles the activity (ref. 11). However, at higher temperatures and higher ethylene oxide concentrations where decontamination times are shorter, the effect of increasing temperatures diminishes.

The polymerization hazard of ethylene oxide also merits concern. In the presence of moisture it is initiated at temperatures of approximately 50°C. As the temperature is increased the degree of polymerization increases. Therefore, it is advisable to use the lowest feasible temperature in a given application. It is usually more desirable to employ a higher concentration of ethylene oxide than to elevate the temperature.

4. Humidity

The moisture level at the site of the bacteria and, therefore, the relative humidity, are significant in the action of ethylene oxide. There are conflicting opinions, however, as to the optimum relative humidity for decontamination. Some sources suggest that a relative humidity of approximately 33 percent is best, and others say that the decontamination efficiency increases with increasing relative humidity. Upon examination of the experiments it appears that the first of these two opinions is correct (ref. 19).

Kay and Phillips (ref. 20) showed an optimal relative humidity in the vicinity of 33 percent, whereas Ernst and Shull (ref. 21), Perkins and Lloyd (ref. 22), and Mayr (ref. 23) presented data to show that sterilizing efficiency increased with increased relative humidity. The methods or test procedures were entirely different and represented diverse condictions. Phillips' optimal low level relative humidity requirement was based on work where microorganisms and their carrier material were allowed to equilibrate with respect to their relative humidity environment. In this case, the optimal relative humidity was determined as 33 percent at 25°C. Efficiency dropped off sharply below 20 percent and dropped more gradually as the relative humidity was increased beyond 40 percent. On the other hand, Ernst and Shull, Perkins and Lloyd, and Mayr tested microorganisms that were equilibrated to a lower relative humidity than that used for the decontamination.

Figure I shows the death rates for spores, equilibrated and tested, at 33, 53, 75 and 98 percent relative humidities (ref. 12). The D values for these curves are 0.8, 1.3, 2.1, and 1.9 hours, respectively. It is apparent that spores were killed more slowly as the relative humidity increased from 33 to 98 percent, but, even so, straight line death rates were obtained over the entire range.

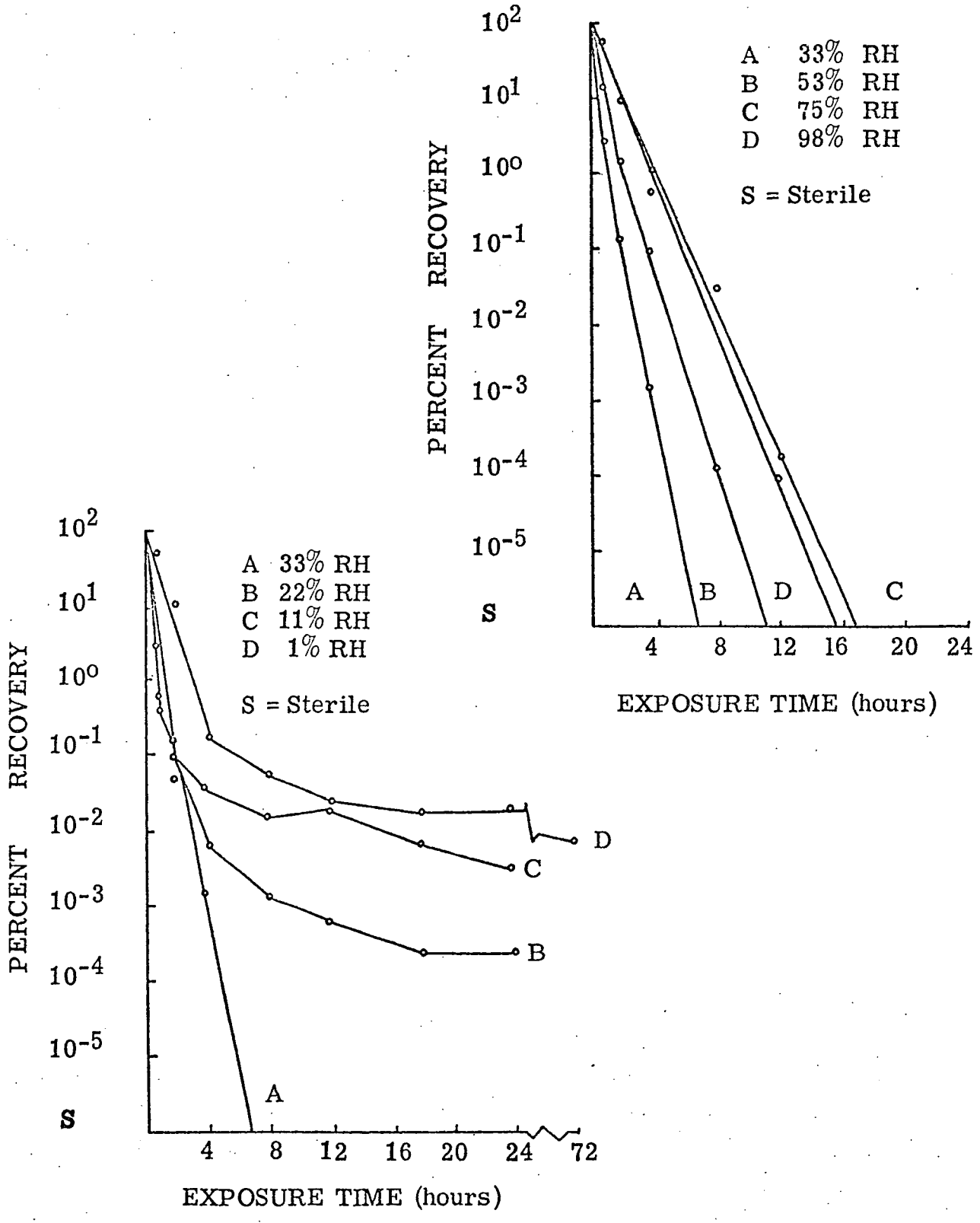

Figure 1. B. subtilis spores on cotton patches exposed to an ethylene oxide concentration of 120 mg/liter at 25°C and the indicated relative humidities, (See Reference 12)

Figure 1 shows the death rates for spores conditioned to relative humidities of 1, 11, 22, and 33 percent and then exposed to ethylene oxide at the same relative humidities (ref. 12). Here the death rates for the spores tested at humidities below 33 percent are not linear like those for higher relative humidities, so no D values can be calculated to describe the entire curve at a given relative humidity. At these low relative humidities, 99.9 to 99.99 percent of the spores were readily killed, but sterility was not obtained even after an exposure of three days. The failure to achieve sterilization cannot be attributed to a low gas concentration because increasing it from 120 to 950 mg/liter did not sterilize the desiccated organisms. Not only is sterilization more difficult at relative humidities below 33 percent, but once microorganisms have been highly desiccated, either chemically or by vacuum, they acquire a resistance that is not completely overcome when the relative humidity is again raised to 33 percent (ref. 11). The results obtained with B. subtilis var. niger spores on porous surfaces, such as cloth or filter paper, reveal that the fastest death rate occurs at a relative humidity of around 33 percent. The need for a higher relative humidity to sterilize impervious surfaces such as glass has been pointed out (ref. 20). To insure sterility of those surfaces, the humidity must be increased to at least 40 percent.

5. Microorganism Age

Older cells appear to be able to withstand longer exposures to severe environments than can young cells (ref. 15). In testing the sterilizing effect of ethylene oxide on B. subtilis var. niger, it was concluded that differences in the resistance of cells of the test strain were due not only to heterogeneity of the population, which included vegetative forms, cells at various stages of germination, and spore forms, but were also related to the age or degree of maturity of spores (ref. 24).

Bomar (ref. 24) inoculated glass beads with B. subtilis and incubated them for 2, 4, 8, 10 and 14 days in sterile desiccators with relative humidities of near zero and 92 percent at 20°C. Zero percent relative humidity was obtained by adding silica gel, 92 percent relative humidity by adding saturated aqueous ammonium phosphate solution. The inoculated beads were then exposed to ethylene oxide in plasma bottles. A flow of ethylene oxide was maintained through the bottles for five minutes at a rate of 1 to 2 liters per minute.

Survival of the spores was studied by two methods. In qualitative evaluation three beads were placed in nutrient broth. If the broth did not become turbid after 2, 4 and 6 days incubation at 30°C, 100 percent sterility had been obtained, while turbidity meant that some of the spores had survived. In quantitative tests, three beads were placed directly in melted medium cooled to 45°C, and after incubation the number of colonies was counted, while another three beads were shaken in 10 ml

distilled water in a test tube, and the number of colonies from this suspension was determined. The cultures were incubated for 2 to 6 days at 30°C.

Table III shows the evaluation of the influence of "maturation" of the spores, i.e., of the transition to different degrees of stabilization of their structure. The initial number of cells per glass bead ranged from 1.6 to 2.6×10^7. The results obtained with zero and 92 percent relative humidity and at temperatures of 20°C and 40°C were practically the same and are shown in Table IV. In Table III, a plus (+) represents better than 99.9 percent reductions or less than 20,000 spores remaining per bead and a zero (0) represents no colonies appearing on the lowest dilution plates.

In the resistance of various microorganisms increases in direct dependance on the spore age, a question arises, concerning the time required for stabilization to be completed and maximum resistance reached. For this reason, tests were made with spores 30, 60, 90 and 120 days old. These were exposed to ethylene oxide under the same conditions. However, no further increase of resistance occurred, which meant that the spores attained their maximal resistance during 30 days under the described conditions. Age distribution of spore populations may also be the cause of the heterogeneity observed in the resistance of spores to biocidal measures in general.

TABLE III

SPORICIDAL EFFECT OF ETHYLENE OXIDE ON BACILLUS subtilis SPORES (From ref. 24)

Age of Spores (In Days)	Time of Exposure of Spores to Ethylene Oxide (In Hours)	Time of Incubation of Exposed Spores (In Days)		
		2	4	6
0	0	+	+	+
	6	0	0	0
2	0	+	+	+
	6	0	0	0
4	0	+	+	+
	6	0	0	0
6	0	+	+	+
	6	0	0	0
8-14	0	+	+	+
	6	+	+	+

TABLE IV

SPORICIDAL EFFECT OF ETHYLENE OXIDE ON BACILLUS subtilis SPORES AT 20°C (From ref. 24)

Incubation of Spores at 92% Relative Humidity (In Days)	Number of Surviving Spores After 6 Hours	
	Ethylene Oxide	Control
0	0	95,000,000
4	0	81,000,000
8	4,700	90,000,000
12	4,600	71,000,000
14	4,750	80,000,000

B. STERILANT MIXTURES

One of the characteristics of gaseous decontaminants is their tendency, when mixed with other agents, to be much more effective than either agent by itself. Effectiveness of mixtures may be enhanced by using them under elevated temperature condictions; however, this procedure is not universally applicable. Evaluation of temperature enhances the effectiveness of alkylating agents but decreases the effectiveness of certain oxidizing agents. In other cases, raising temperature may result in reduced absorption of the toxic agent by the cell, and may cause undesirable chemical reactions of the agents or polymerization (ref. 15).

Three substances that have been tested in mixtures with ethylene oxide are methyl bromide, dimethyl sulfoxide, and formic acid. The activity of the ethylene oxide-methyl bromide mixture, (60 percent ethylene oxide and 40 percent methyl bromide) at 25°C, was not significantly different from ethylene oxide alone against B. subtilis var. niger spores on cottom fabrics, glass and fiberglass, at 1 and 33 percent relative humidity; or against S. epidermidis under extremely dry conditions (1 percent RH). However, with S. epidermidis at 33 percent relative humidity, over 99 percent of the population was killed within 7.5 minutes with the ethylene oxide - methyl bromide mixture, while 30 minutes were required to attain this reduction with either substance alone (ref. 25). The increased activity of the mixture did not appear to be a synergistic effect but an additive one, because the activity of

methyl bromide alone was appreciable under these conditions, and not significantly different from ethylene oxide alone. In those instances where the sporicidal activity of the mixture did not differ from ethylene oxide alone, the affect of methyl bromide alone was found to be slight and significantly less than that of ethylene oxide.

The sporicidal activity of the ethylene oxide - methyl bromide mixture was also determined against B. subtilis var. niger spores on cloth patches, both uncovered and sealed within polyethylene or polyvinyl chloride plastic envelopes (ref. 26). No difference in activity between the mixture and ethylene oxide alone was observed against spores not protected by plastic. However, the mixture was measurably more effective than ethylene oxide alone against spores sealed in polyvinyl chloride, and slightly more effective against spores sealed in polyethylene. Methyl bromide apparently promotes the rate of ethylene oxide pentration, at least through some plastics, thus shortening the exposure period required for decontamination.

Tests were conducted by Spiner (ref. 27) to determine whether or not there was an increase in the sporicidal activity of ethylene oxide gas when it was mixed with dimethyl sulfoxide. The sporicidal activity of dimethyl sulfoxide vapor appeared measurable at saturation concentration, but its low volatility made these measurements difficult. Exposure to ethylene oxide in the presence of dimethyl sulfoxide vapor seemed to cause an increase in the number of spored killed. However, too few data were collected to prove statistically that the addition of dimethyl sulfoxide vapor significantly enhanced the sporicidal activity of ethylene oxide.

Data by Kaye (ref. 28) indicate that formic acid, applied either as vapor or in solution, catalyzes the sporicidal action of ethylene oxide vapor or solution. The concentration of formic acid, within certain limits, determines the extent of this potentiation whose nature is such that one can either use less ethylene oxide, or shorter exposure time, to obtain the desired level of decontamination. The advantage of this process may lie not so much in the decreased dosage, per se, but in the resulting minimization of the absorption of ethylene oxide by porous or elastometric materials. The shorter the contact time, or the lower the ethylene oxide concentration to which such materials are exposed, the less the amount of chemical absorbed.

C. PENETRATING ABILITY

All materials in which microorganisms are imbedded protect the organism, to some degree, from the lethal effects of ethylene oxide. In most cases, this protection probably results from the reduced diffusion of the ethylene oxide and water in the material rather than from any chemical reaction between the material and the ethylene oxide. The degree of protection afforded under various circumstances, including spores in solutions (ref. 12) in plastic bags, (ref. 12 and 29) and between mated surfaces, (ref. 30) has been experimentally determined.

Tests on bacterial spores suspended in oil and water showed that ethylene oxide gas will dissolve in and diffuse through the media to kill the microorganisms (ref. 12). Both liquids act as protective

materials in that they increase the exposure time required for sterilization. The time required to sterilize as a function of depth is shown in Table V. Various depths of olive oil and water in test tubes containing B. subtilis var. niger spores were used. These were exposed for 24 hours in a chamber containing 450 mg/liter of ethylene oxide at 25°C. The olive oil was sterilized to a depth of 1 cm, but at greater depths less kill was obtained. Water was sterilized to a depth of 1.8 cm, and again less kill was obtained as depth increased.

TABLE V

PERCENT KILL OF B. subtilis var. niger SPORES IN OLIVE OIL AND WATER[a] (From Ref. 12)

LIQUID		PERCENT KILL	
Depth, cm	Volume, cm	Olive Oil	Water
0.8	1	100	100
1.8	2.5	98	100
3.5	5	91	88
6.8	10	57	55

a. Exposed to 450 mg/liter of Ethylene Oxide for 24 hours at 25°C

The influence of thickness of polyethylene film on the sporicidal activity of ethylene oxide is shown in Table VI. The experiment yielding this data utilized spores of B. subtilis var. niger dried on hygroscopic and nonhygroscopic surfaces. The surfaces were then enclosed in 2, 4, 6 or 20 mm thick polyethylene films and exposed to an ethylene oxide concentration of 600 mg/liter at 54.4°C and 50 percent relative humidity (ref. 29). Table VI gives the decimal reduction values associated with these various conditions. Survival data are similar for the spores in the 2, 4, and 6 mm films. The use of 20 mm film, however, greatly increased the time required to kill the spores under the exposure conditions.

TABLE VI

DECIMAL REDUCTION TIMES OF B. subtilis var. niger SPORES DRIED ON SURFACES ENCLOSED IN POLYETHYLENE FILMS[a] (From Ref. 29)

Thickness of polyethylene film	Nonhygroscopic surface	Hygroscopic surface
mm	min	min
0[b]	3.75	2.15
2	3.00	3.40
4	3.30	3.45
6	3.10	3.84
20	15.00	13.10

a. Values are expressed as D at 54.4°C, 600 mg of ethylene oxide per liter, and 50% RH.
b. Glassine envelope.

It is hypothesized that water molecules carry ethylene oxide to reactive sites (ref. 19). In practice, either water or ethylene oxide increases the permeation of the other through plastic films, depending on their polar or nonpolar character. The fact that ethlene oxide acts as a carrier for moisture through nonpolar, and normally hydrophobic films having low moisture permeations, was observed by Ernst and Doyle (ref. 19). When a sealed polyethylene bag was placed in a sterilizer conditioned to 54.4°C in a high relative humidity, little if any moisture diffused through the polyethylene film. However, on addition of ethylene oxide to this system, globules of water were found on the inside of the bag, indicating that ethylene oxide acted as a carrier for the diffusion of moisture through the polyethylene film. Conversely, water aids the permeation of ethylene oxide through polar type films, e.g., nylon and cellophane, which normally allow water to readily permeate but are slow to diffuse ethylene oxide.

A comparative study was undertaken by Portner (ref. 30) to determine whether there is an appreciable difference in the sterilizing effectiveness of dry heat or of ethylene oxide gas, upon microbial contamination located between mated surfaces, in contrast to the contamination located on the external surface of a material. An aqueous spore suspension of B. subtilis var. niger was used to inoculate each sample. The dry heat tests were conducted at 105°C and 125°C. The gaseous sterilization tests employed an ethylene oxide concentration of

approximately 337 mg/liter in an ethylene oxide-Freon mixture at ambient temperature.

The results of this study are summarized in Tables VII and VIII. The mated surface configurations exposed in dry heat tests were:

- First test - Roundhead steel machine screws ($\frac{1}{4}$ -20 and 1 inch long) with appropriate nuts, stainless stell strips 1 x 2 x 1/16 inches); and glass slides (1 x 2 x 3/64 inches)
- Second test - Glass squares (1 x 1 x 3/64 inches)
- Third test - Stainless stell squares ($1\frac{1}{2}$ x $1\frac{1}{2}$ x 5/64 inches) and glass squares (1 x 1 x 3/64 inches)

The ethylene oxide test used the same materials as the third dry heat test.

TABLE VII

THE EFFECTIVENESS OF DRY HEAT UPON
B. subtilis var. niger SPORE CONTAMINATION
LOCATED ON AND BETWEEN SURFACES
OF VARIOUS MATERIALS (From ref. 30)

Dry Heat Tests	Temp.	Material and Quantity	Exposure to Heat Hours	Number Spores/Sample Location of Contamination On Surface	Between Surfaces
1st	125C	1 screw and 3 nuts	0	519,000	519,000
			1	194	204
		2 stainless steel strips	0	704,000	704,000
			1	209	349
		3 glass slides	0	826,000	826,000
			1	206	189
2nd	105C	2 glass squares	0	887,000	887,000
			4	50,100	67,300
			24	1	7
			48	0	0
		6 glass squares	0	887,000	887,000
			4	59,400	79,000
			24	2	1
			48	0	0
3rd	125C	2 stainless steel squares	0	460,000	460,000
			1	1	6
		2 glass squares	0	870,000	870,000
			1	27	25

Note: For the 1st tests, each entry is an average of 4 samples.
For the 2nd tests, each entry is an average of 16 samples.
For the 3rd tests, each entry is an average of 12 samples.

* All flat surfaces were held together with binder clips except the stainless steel squares which were bolted on all four sides.

TABLE VIII

THE EFFECTIVENESS OF ETHYLENE OXIDE GAS *
UPON B. subtilis var. niger SPORE
CONTAMINATION LOCATED ON AND BETWEEN
STAINLESS STEEL OR GLASS SURFACES (From Ref. 30)

Material	Exposure to ETO	Number Spores/Surface Location of Contamination	
	Hours	On Surface	Between Surfaces
Stainless Steel	0	331,000	331,000
	4	5	44
Glass	0	859,000	859,000
	4	4	5

Note: Each entry is an average of 12 samples.

* 337 mg/liter of air.

In all three series of tests summarized in Table VII, dry heat appeared as effective against spore contamination located between mated surfaces as against spore contamination located on the exterior surface of the various materials tested. Table VIII shows that in general ethylene oxide gas was as effective against spore contamination located between mated surfaces as it was on the exterior surfaces of steel or glass. For both dry heat and ethylene oxide tests, exposure periods were selected that would reflect a decrease in population, but not sterility, so that numerical comparisons could be made.

D. RELIABILITY

Studies of the sterilizing ability of ethylene oxide were made using dustborne spores of B. subtilis contaminating surfaces with averages of about 5×10^4 organisms per square foot (ref. 17). Gas concentrations up to approximately 3200 mg of ethylene oxide per liter; exposure periods from 1 to 24 hours; temperatures from 26.7 to 48.9°C; and relative humidities from 10 to 80 percent were employed. Although gas concentrations considerably in excess of those normally recommended for sterilization purposes were used, a significant number of the sterilization cycles did not kill exposed populations of the B. subtilis. A summary of unsuccessful sterilization attempts at near optimal conditions, i.e., 48.9°C and 50 percent relative humidity, is shown in Table IX.

TABLE IX

NUMBER OF UNSUCCESSFUL STERILIZATION TRIALS AGAINST DUSTBORNE SPORES OF B. subtilis at 50 PERCENT RELATIVE HUMIDITY AND 48.9°C (From Ref. 17)

ETO CONCENTRATION (mg/liter)	TOTAL NUMBER OF TRIALS	UNSUCCESSFUL TRIALS	
		(Number)	(Percent)
400	12	5	42
700	8	2	25
1000	35	7	20
1600	11	2	18
3200	6	2	33
TOTALS	72	18	24

The results indicate that sterility was obtained in the majority of trials under the various test conditions; however, a significant number of sterilization cycles failed. Most of the exposure periods were of 4, 18, and 24 hours duration; tests limited to 1 hour exposures were primarily with the high concentrations of ethylene oxide. Sterilization was not achieved in a total of 24 percent of trials (Table IX). Excluding exposure periods of less than 4 hours duration, which is considered an acceptable exposure period for moderate levels of gas concentration, the failure rate was still 15 percent.

High concentrations of gas did not preclude sterilization failures. Viable spores were recovered after exposure to 1600 mg/liter of ethylene oxide for 24 hours. Even at the 3200 mg/liter level of gas concentration, with exposure periods of 4 hours duration, sterilization of the test surfaces was not accomplished in all runs, as shown in Table X. The higher gas concentrations are considerably in excess of those normally considered necessary for sterilization with gaseous ethylene oxide. The results indicate that high concentrations of ethylene oxide apparently do not increase the reliability (note: this is consistent with the findings cited in section III. A. 1.).

TABLE X

UNSUCCESSFUL TRIALS OF ETHYLENE OXIDE GAS AGAINST B. subtilis AT 50 PERCENT RELATIVE HUMIDITY AND 48.9°C (Ref. 17)

ETO CONCENTRATION	EXPOSURE PERIOD (hours)							
mg/liter	1	2	3	4	5	6	18	24
400	x	x	x	x	x	x	x	x
700						x	x	x
1000	x	x	x			x	x	x
1600	x	x	x		x	x	x	x
3200	x	x		x				

Again considering Table X, it is evident that increasing the duration of exposure to as long as 24 hours will not increase the dependability of sterilization of B. subtilis by ethylene oxide gas. Skips were obtained at practically all time intervals tested, and no particular exposure period yielded consistently better results.

Relative humidity was maintained at 50 percent or less for most of the tests. Only a few trials were conducted at higher moisture levels. The results showed that sterilization was more consistent at 50 percent than at lower humidities. Under conditions of low relative humidity there was little, if any, sterilization regardless of concentration, time, or temperature. With relative humidities at the 10 percent level, approximately 90 percent failures were observed for all concentrations and exposure periods tested.

Variations in temperature in the 26.7 to 49.9°C range resulted in only small differences in the number of sterilization failures. These differences, however, suggested that increases in temperature, within the range practical for ethylene oxide (i.e., approximately 20°C to 60°C) do not markedly increase the sterilization efficiency of the gas against dustborne spores of B. subtilis.

Decontamination, or reduction of microorganism population, as opposed to sterilization, should be the goal of the ethylene oxide treatment. With reasonable values of the environmental parameters discussed previously, a three order of magnitude reduction of the surface contamination can be achieved without undue difficulty. Sterility, however, as demonstrated above, may be difficult or even impossible to achieve.

IV. METHODS OF APPLICATION

SPECIAL HANDLING

Ethylene oxide should be handled with all precautions generally observed for flammable materials. Although in the liquid state it is soluble in water, it will continue to burn if ignited unless the dilution is approximately 22 volumes of water to one, or greater (ref. 9). Liquid ethylene oxide is quite stable to explosion initiators but, as a vapor, it may undergo rapid decomposition if an explosion is initiated. This action is accompanied by the liberation of a considerable amount of energy, and, if in a confined space, will produce an increase in pressure by a factor of 16 to 50 (ref. 9). The pressure ratio varies with the ratio of volume to surface of the containing vessel. However, as noted in section II, this explosion hazard can be eliminated by diluting the ethylene oxide concentration to approximately 10 percent in an inert gas such as Freon or carbon dioxide (ref. 11). Contact of ethylene oxide with substances that catalyze polymerization or decomposition should be avoided. Therefore, steel or stainless steel construction is desirable for piping, instrument leads, storage tanks, and other equipment, handling ethylene oxide.

V. EFFECTS ON PERSONNEL

Contact with ethylene oxide, either through inhalation or direct skin exposure, can have adverse effects on personnel. The acute inhalation toxicity of ethylene oxide has been reported as moderate, being about the same as ammonia gas (ref. 31); 50 parts per million has been recommended as the maximum allowable concentration for prolonged human exposure (ref. 11). Joyner (ref. 32) found no evidence of chronic toxicity among plant workers who had experienced a mean ten year exposure to concentrations of approximately 5 to 10 parts per million. Concerning short terms exposures, there is information indicating that a person can work in an atmosphere containing 250 parts per million, in single exposures of one hour duration, without being harmed (ref. 33). Fortunately, one of the first symptoms noticed on overexposure to ethylene oxide is irritation to the eyes and nose, which helps to warn of the presence of a hazardous concentration. Other symptoms that may occur are nausea, vomiting, and mental disorientation. No reports of death due to ethylene oxide inhalation have been found.

The second toxic effect of ethylene oxide lies in the fact that its vapors are vesicant to skin if contact is prolonged, particularily if aqueous solutions are spilled on clothing or other absorbent materials which are in continuing contact with the skin. (See refs. 11, 31, and 34.)

Cases have been reported where laboratory workers' feet were badly blistered when they wore rubber shoes which had recently been exposed to ethylene oxide. If spilled on exposed skin, ethylene oxide usually evaporates too rapidly to cause serious damage. It can, however, produce eye burns. Vapors have also been found to produce skin irritations and burns because of absorption by perspiration on the body.

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