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Simple Improvised Chambers for Gas Sterilization with Ethylene Oxide

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This paper describes readily available simple exposure chambers in which material or equipment may be sterilized by ethylene oxide gas. Although specialized automatic equipment is now available for such purposes (Perkins, 1956; Stryker, 1958), occasions may arise where it it desirable (a) to adapt laboratory equipment, such as a standard autoclave so that it can be used with ethylene oxide, without interfering with its regular use, or (b) to have cheap, mobile equipment that can be readily transported to outside locations where one has material to sterilize. The present availability of nonflammable ethylene oxide mixtures packaged in light weight aerosol bomb type cans makes it quite feasible to utilize such simple exposure chambers and, as the data presented show, material can be readily sterilized in such devices.

Ethylene oxide is a gas at room temperature and 760 mm Hg pressure, but is readily liquefied because of its relatively high boiling point (10.8 C). Keeping the liquefied compound in simple containers presents a safety hazard in both storage and use, since the vapor is highly flammable in air in any concentration between 3 and 80 per cent by volume. For many years the compound has been commercially available, for fumigation or sterilization purposes, in combination with carbon dioxide,[1] an inert diluent which renders the mixture nonflammable when present in air in any proportion (Jones and Kennedy, 1930). Because of the high internal pressure exerted by the carbon dioxide, this mixture can be contained only in heavy steel cylinders which are both costly and awkward to handle.

Numerous improvised experimental setups have been described in the literature on the use of ethylene oxide, alone or in combination with carbon dioxide, to sterilize materials or equipment. For example, Yesair and Williams (1942) sterilized spices under vacuum in an aluminum kettle containing ethylene oxide gas and no inert diluent. Kaye and Surkiewicz (1952) sterilized equipment in bags or under tarpaulins made of gas tight coated fabrics. The pure ethylene oxide gas was admitted into the system by crushing glass ampules of the liquid chemical or by releasing it from metal cylinders. Fulton and Mitchell (1952) used an ethylene oxide-carbon dioxide mixture when experimenting with such improvised chambers as (a) a wooden box, (b) a hypochlorite bleach drum, and (c) a segment of an automobile rubber inner tube in which footwear was sterilized. Newman *et al.* (1955) sterilized articles in a standard autoclave by use of an ethylene oxide-carbon dioxide mixture admitted to 20 lb pressure from heavy cylinders. In all of these experiments either a flammability hazard existed when using undiluted ethylene oxide or a second problem was created when admitting an ethylene oxide-carbon dioxide mixture under high pressure from awkward heavy cylinders into small confined spaces which would not hold all of the vapor should the entire contents of the cylinder be accidently released.

The sterilant mixture used in the experiments described herein was developed for the U.S. Army Chemical Corps by the Department of Agriculture (Haenni *et al.*, 1959). It contains 11 per cent (by weight) ethylene oxide, 44.5 per cent dichlorodifluoromethane, and 44.5 per cent trichloromonofluoromethane. It is packaged[2] in 16 oz low pressure aerosol cans containing 66 g (75 ml) of ethylene oxide. Each aerosol can is

[1] Carboxide; product of Carbide and Carbon Chemical Company, New York, New York.

[2] Pennsylvania Engineering Company, Philadelphia, Pennsylvania.

fitted with a 1-in. flat cap to which a needle valve with a can holder and hollow needle[3] is attached to release the sterilant mixture (figure 1).

The substitution of fluorinated hydrocarbons for carbon dioxide as a diluent permits the ethylene oxide to be packaged as a nonflammable mixture in light weight aerosol-bomb type cans. The use of the fluorinated compounds with their higher molecular weights also permits a higher concentration of ethylene oxide in the vapor state than can be achieved with the carbon dioxide mixture. Although the weight percentage for nonflammable formulations are approximately the same in the two mixtures, the molar percentage of ethylene oxide and hence the volume percentage of its vapor is about 2.7 times as high with the fluorinated hydrocarbon mixture as it is with Carboxcide.

The use of an ethylene oxide-fluorinated hydrocarbon mixture was first reported by Skeehan *et al.* (1956), in their paper on the sterilization of ophthalmic instruments in an autoclave simply converted for the purpose by the Fort Detrick investigator in this joint study. Since then, at least two ethylene oxide-fluorinated hydrocarbon mixtures have become available on the commercial market.[4] Each of these mixtures contains approximately the same amount of ethylene oxide as that used for the work reported herein but contains different proportions of the two fluorinated hydrocarbon diluents.

Three quite simple exposure chambers, which utilize the low-pressure nonflammable ethylene oxide mixture, are described below with data on their performance.

[3] Can-O-Gas Valve; manufactured by the Virginia Smelting Company, West Norfolk, Virginia. Dual Charging Can Valve; manufactured by the Superior Valve and Fittings Company, Pittsburgh, Pennsylvania.

[4] Cryoxcide; product of American Sterilizer Company, Erie, Pennsylvania. Steroxcide; product of Wilmot Castle Company, Rochester, New York.

Figure 1. Can holder, aerosol can, and holder attached to can in operating position.

Materials and Methods

Exposure Chambers

Autoclave. The autoclave was selected as an ethylene oxide sterilization chamber because of its universal availability and ease of modification. The autoclave was modified to permit the use of either steam or ethylene oxide gas. A valve was installed on each pipeline attached to the autoclave if the line was not equipped with a valve. By means of these valves all exhaust and drain lines could be closed to make the autoclave a sealed chamber. A pipe "tee" was installed on one of the exhaust lines between the cutoff valve and the chamber. A needle valve with can holder and hollow needle was attached to the pipe "tee" with ¼ in. (o.d.) copper tubing. The can holder was attached in such a way that it held the can of sterilant in an inverted position (figure 2). This position is mandatory as the cans are not equipped with eductor tubes. The volume of the autoclave used in these experiments was 120 L so that the injection of 66 g of ethylene oxide gave a concentration of 550 mg of the oxide per L of space.

The procedure for sterilizing materials in the autoclave simply involved placing the contaminated items in the autoclave, closing the door and all valves and then admitting the sterilant. Air was not evacuated from the autoclave, nor was the temperature raised before admitting the ethylene oxide. The sterilant mixture

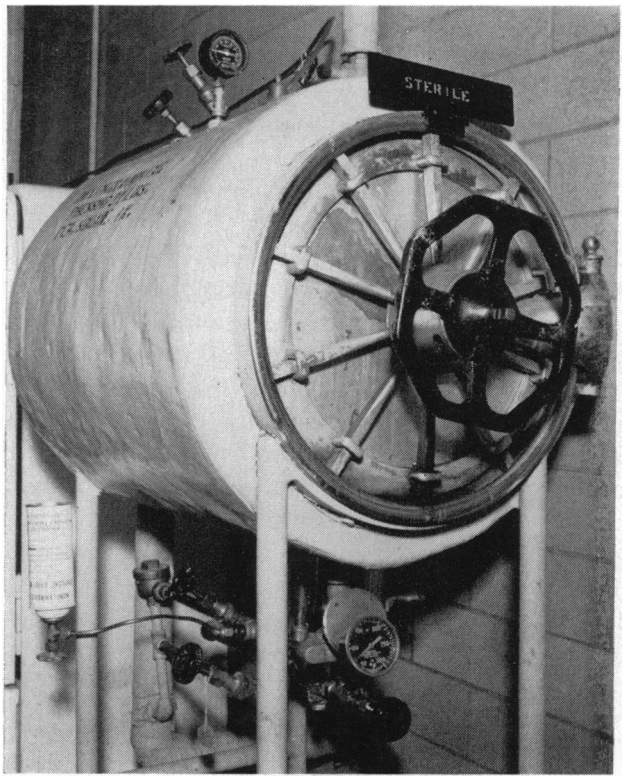

Figure 2. Autoclave modified for gaseous sterilization

was admitted by attaching the can to the holder, screwing the can down until the hollow needle pierced the cap, and then opening the needle valve.

Drums. The two drums tested were unlined steel drums of flash welded seam construction. The primary difference between the two drums was the method of closure. The lid of one drum was sealed by a metal ring with a lever type lock known as Quik-Lox. The second drum also was equipped with a steel ring but the ring was tightened with a nut and bolt. Both drums were satisfactory as ethylene oxide sterilizing chambers.

Each drum was modified by cutting two, ½-in. holes in the lid. To each hole was brazed a ¼- to ⅜-in. pipe reducer. A pressure gauge was attached to one fitting (figure 3) and a needle valve, with can holder and an inductor tube, was attached to the second fitting. A series of tests was made to determine the maximum pressure to be encountered when the sterilant mixture was added. The maximum drum pressure encountered was 6 psig; above this pressure, leakage occurred around the drum head gasket. Preliminary experiments indicated that more consistent bacteriological results were obtained if the drum was fitted with an inductor tube. This tube permitted the halogenated hydrocarbon-ethylene oxide formulation to be released in the bottom of the drum. As the formulation expanded within the drum, pressure increased and air was forced out around the gasket. If no inductor tube were present, more ethylene oxide would be forced out of the drum and a longer exposure time would be required.

The adequacy of the drum to serve as a container for ethylene oxide sterilization was found to depend upon the sealing qualities of the drum head gasket. Three types of gaskets were tested. One, made of 3/16-in. thick sponge rubber was used only once or twice before it became compressed and thus nonserviceable. The second gasket was a hollow, hemispherical, seamless, rubber or pliable plastic gasket made with four longitudinal ridges along its flattened side. It was used eight times with no observable loss of sealing quality. The third gasket, a round hollow rubber type, was used three times with no apparent loss in serviceability. The 55-gallon drum has a volume of approximately 210 L and therefore the addition of 66 g of ethylene oxide gives a theoretical concentration of approximately 320 mg of the oxide per L of space. Actually, the concentration of ethylene oxide within the drum would be somewhat less than the theoretical 320 mg per L since some of the vapor will be forced out of the drum with the air when the pressure surpasses 6 psig upon admitting the sterilant to the system.

The procedure used to sterilize material in the drum was somewhat similar to that used with the autoclave. Clothing and cloth patches contaminated with *Bacillus subtilis* var. *niger* (*B. globigii*) spores were placed in the drum, the drum closed, and the ethylene oxide can attached to the holder. The can was screwed down on the hollow needle and the sterilant mixture admitted by opening the needle valve. The drum was then rolled on its side a few times to insure a homogenous distribution of the gas throughout the container. The drum was placed in an upright position during the sterilization period.

Polyethylene bag. The simplest device used for ethylene oxide sterilization was a large polyethylene bag. Preliminary tests with various plastics showed that a 4 to 6 mil thick polyethylene film would retain ethylene oxide gas for a period of time sufficient to achieve sterilization. Bags made from this polyethylene

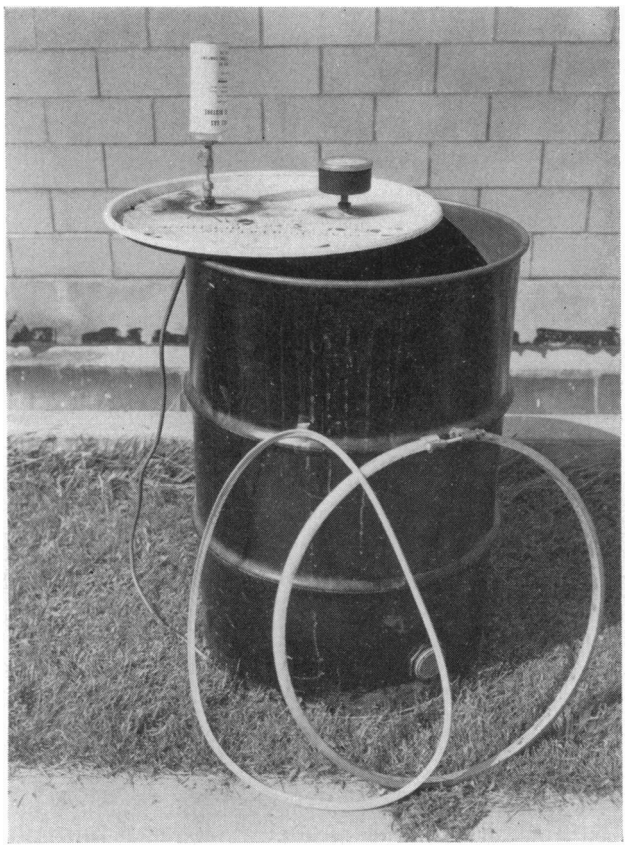

Figure 3. Drum modified for gaseous sterilization

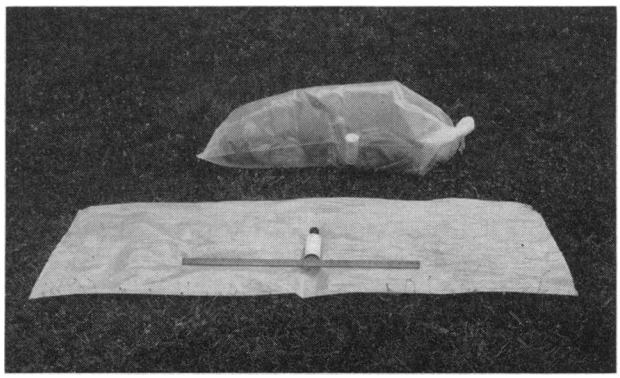

Figure 4. Polyethylene bags, empty and used as a sterilizing container.

film were selected because they were commercially available as chemical drum liners, could be used repeatedly, stored for a long period of time, and re-used without the bag losing its flexibility and gas holding qualities. The bags used in these tests were 6 ft long and 2½ ft wide when flat (figure 4). They were constructed of tubular polyethylene and had a seam only at the bottom. The bag when closed had a volume of approximately 145 L and therefore the addition of 66 g of ethylene oxide gave a concentration of approximately 450 mg of the oxide per L of space.

The procedure used to sterilize the material in the bag was also quite simple. The clothing or equipment was placed in the bag. A needle valve with a can holder was attached to an ethylene oxide container, the valve closed and the can screwed down until the hollow needle punctured the cap. The can with the needle valve attached was placed in the center of the bag but to one side. The bag was closed by twisting approximately 2 ft of the neck, folding it over on itself and tying it tightly with a piece of string. The closed bag was positioned on its side and the can of sterilant grasped from the exterior of the bag and the valve opened while holding the can in an inverted position. When the bag was used as a container in which to sterilize clothing, it was rolled through an arc of 360° a few times to insure a thorough distribution of the disinfectant. When sterilizing delicate equipment, of course, the bag could not be rolled. Following the rolling procedure the bag was stored undisturbed at room temperature for the desired exposure period.

TABLE 1

Recovery of Bacillus subtilis var. niger spores from instruments before and after exposure to ethylene oxide

Exposure Time	Instrument	Polyethylene Bag		Instrument	Autoclave	
		No. of spores recovered*			No. of spores recovered*	
		Before treatment	After treatment		Before treatment	After treatment
hr						
2	Hygrothermograph	110,000	2,140	Centrifuge	325,000	53
2	"	830,000	20,175	"	442,500	225
4	"	159,000	0	"	460,000	0
4	"	19,500	0	"	24,350	0
6	"	46,500	0	"	58,500	0
2	pH meter	167,500	1,064	Colony counter	20,000	0
2	"	985,000	18,000	"	1,085,000	0
4	"	290,000	0	"	660,000	0
4	"	310,000	0	"	99,000	0
6	"	395,000	0	"	104,000	0

* Average of two samples.

TABLE 2

Recovery of Bacillus subtilis var. niger spores from cloth patches before and after exposure to ethylene oxide

Container	Exposure Time	Control No. of Organisms per Patch	No. of Organisms Recovered from Cloth Patches after Exposure*		
			Top	Middle	Bottom
	hr				
Bag	4	5,000,000	71	4	11
Bag	4	5,000,000	478	183	270
Bag	4	5,000,000	1,119	356.5	83.5
Bag	4	5,000,000	397.5	176	85
Bag	6	9,400,000	0	0	0
Bag	6	9,400,000	0	0	0
Drum	4	5,000,000	339	183	270
Drum	4	9,000,000	20	10	18
Drum	6	3,700,000	0	0	0
Drum	6	9,400,000	0	0	0

* Average of two samples.

Biological Methods

The adequacy of the container to serve as an ethylene oxide sterilization chamber was determined by its ability to retain the oxide long enough to sterilize cloth patches or instruments contaminated with a viable spore suspension of *B. subtilis* var. *niger*. The degree of decontamination obtained on the test objects served as an index of the decontamination expected for similar items.

Cotton twill coveralls were used for bulk in these tests. The contaminated cloth patches were placed strategically through the clothing in the respective chambers so that it could be assured that the ethylene oxide penetrated the bulky clothing. Samples were taken before and after the sterilization procedure and assessed using the standard bacteriological pour plate technique.

RESULTS

Table 1 shows that the laboratory instruments which were heavily contaminated with bacterial spores were sterilized in the autoclave or polyethylene bag after 4 hr exposure at room temperature to a theoretical concentration of 550 and 450 mg of ethylene oxide per L of space, respectively. Although 2 hr exposure sterilized some contaminated sites on the instruments, viable spores were still recovered from other sites.

Table 2 shows that heavily contaminated clothing would require about 6 hr exposure to ethylene oxide before sterility would be obtained in a polyethylene bag or drum.

DISCUSSION

It is evident from the results presented that the autoclave, steel drum, and polyethylene bag can serve as chambers in which to sterilize laboratory equipment or clothing with ethylene oxide. The fact that such simple equipment can be used does not signify that there may not be many occasions where more elab-

orate permanent types of chamber installations may be preferable, particularly when the volumes of material being routinely sterilized are large. Neither is the ethylene oxide sterilizing method recommended as a replacement for the cheaper routine steam or heat sterilization methods where they can be used. The ethylene oxide system does, however, furnish a means of sterilizing nonautoclavable heat and moisture sensitive materials in the laboratory, or of sterilizing almost any type of material in the field where steam is unavailable.

Materials may be maintained in a sterile condition after decontamination if they are wrapped in paper before exposure to the ethylene oxide. The oxide readily penetrates the paper and sterilizes the items while the paper in turn prevents recontamination.

Materials which are heavily contaminated with resistant bacterial spores will require an exposure to 300 to 500 mg ethylene oxide per L of air for about 6 hr to insure sterilization at room temperature (25 C). Since each 10 C change in temperature changes the effectiveness of the chemical by a factor of 2 to 3 (Phillips, 1949), attempts to sterilize outdoors in cold weather should be avoided.

No effort was made to evacuate the drum or autoclave before admitting the ethylene oxide mixture. A vacuum is not necessary to achieve penetration of ethylene oxide through porous materials since this highly volatile gas diffuses rapidly through many layers of fabric or other permeable materials (Phillips, 1957). Also it had been noted that, when air was evacuated from a chamber before admitting ethylene oxide vapor, sterilization often became more difficult, and sometimes was not achieved in the expected time interval. This has been attributed to the removal of moisture as well as air when the chamber was evacuated. Apparently some moisture is necessary for the killing process to proceed rapidly (Yesair and Williams, 1942; Kaye and Phillips, 1949).

It must be emphasized that the can of ethylene oxide-halogenated hydrocarbon mixture must be held in an inverted position to insure a rapid release (within several minutes) of the sterilant when the can is opened. The can is not equipped with an eductor tube and, therefore, if the can is opened while in an upright position, it will require a much longer time (about 24 hr) to dispense the entire contents.

Care must be exercised when using ethylene oxide and liquid halogenated hydrocarbon mixture since both liquid ethylene oxide and liquid trichloromonofluoromethane are excellent solvents for certain plastics. Therefore, it is imperative that droplets of the liquid disinfectant mixture, before they have an opportunity to vaporize as they emerge from the can, not be permitted to contact certain materials. Ethylene oxide and trichloromonofluoromethane evaporate quite rapidly after dissemination and once in the gaseous state they are noncorrosive. It is advisable to provide a shield or baffle over the port through which the sterilant mixture enters the autoclave or the drum. When sterilizing equipment in a bag, it is desirable to direct the effluent from the dispenser into a towel or rag to protect equipment which might be damaged by the liquid sterilant or propellant.

Summary

A simple ethylene oxide dispenser system and three easily devised exposure chambers are described. The dispenser is a 16 oz, low-pressure aerosol can containing 11 per cent (by weight) ethylene oxide, 44.5 per cent dichlorodifluoromethane, and 44.5 per cent trichloromonofluoromethane. The aerosol cans are fitted with a 1-in. flat cap to which a needle valve with a can holder and hollow needle can be attached to release the sterilant mixture. This system was adapted for use with an ordinary steam autoclave, a 55 gallon open-head steel drum, or a simple large polyethylene bag. Any one of these devices can be used as an ethylene oxide sterilizing chamber in the laboratory and the drum and polyethylene bag are suitable for use in the field.

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