Beta-Propiolactone Vapor as a Disinfectant[1]

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Received for publication, March 14, 1958

Despite recently revived interest in gaseous disinfection or sterilization, only two compounds, formaldehyde and ethylene oxide, have been used to any great extent for this purpose. As discussed by Phillips (1957) in a review of gaseous sterilization, several other compounds have been mentioned in the literature as possessing bactericidal properties in the vapor state but have been put to little, if any, practical application.

Formaldehyde had widespread application in the early part of this century for disinfecting sick rooms following occupancy by a person suffering from a contagious disease. It is seldom used for this purpose now, although it still has certain industrial applications as a vapor-phase disinfectant, particularly for large inclosed spaces. Ethylene oxide has come into use only within the last decade. The use of this compound, usually contained in mixtures with carbon dioxide or fluorinated hydrocarbons to reduce its flammability, has permitted the sterilization of many types of materials hitherto considered nonsterilizable because of heat or moisture sensitivity. These materials include plastics, wool, leather, pharmaceuticals, and articles such as notebooks, delicate laboratory equipment, and medical instruments among many others. In general, gaseous disinfection techniques are not proposed to displace other routine standard methods such as steam or chemical solution sterilization, but rather to meet many peculiar problems arising when sensitive materials, for one reason or another, need to be sterilized.

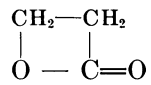
Recent activity in gaseous sterilization has been confined largely to the exploitation of new applications of formaldehyde and ethylene oxide, particularly the latter, rather than to a search for additional compounds with different physical or chemical properties. A continuing program of search has been conducted in these laboratories; out of this program β-propiolactone has emerged as a compound with high biological activity in the vapor state.

β-Propiolactone more nearly resembles formaldehyde than ethylene oxide in its germicidal properties and possesses a number of advantages over formaldehyde as a gaseous sterilant, particularly with respect to increased antimicrobial activity and lessened persistency. To date, the microbiological activity of this compound has been reported only for aqueous solutions (LoGrippo *et al.*, 1955 and Hartman *et al.*, 1955) and not for the vapor.

β-Propiolactone is a colorless liquid at room temperature with the following pertinent physical properties:

Boiling point	155 C (decomposition)
Melting point	−33.4 C
Density 20/4 C	1.1490
Vapor pressure (25 C)	3.4 mm Hg
Water solubility (25 C)	37% (by volume)

The structural formula of β-propiolactone is:



It is characterized by a slightly sweetish odor which is irritating and has lachrymatory properties even at low concentration. The lactone hydrolyzes readily in water to form hydracrylic acid. Data collected in these laboratories, and shown in table 1, point out that the rate of hydrolysis varies with the temperature. The hydrolysis rate for each temperature is expressed as the half-life which is the time required to reduce the concentration of β-propiolactone to 50 per cent.

Purified (99 per cent) or commercial grade (96 per cent) β-propiolactone may be stored at 4 C for at least 3 years without appreciable change. However, if stored at higher temperatures, for example, 54 C, it undergoes polymerization within 6 to 8 weeks. Liquid β-propiolactone exhibits chemical properties typical of lactones in general; it reacts readily with organic compounds containing available amino, carboxyl, sulfhydryl, and hydroxyl groups (Szilagyi *et al.*, 1954). In addition, inorganic salts, acids, or alkalis catalyze the polymerization of the liquid or react with it to yield a new product (Gresham *et al.*, 1948a, b).

Some studies have been reported on the use of aqueous solutions of β-propiolactone as a virucide and bactericide. Hartman *et al.*, (1955) have shown that β-propiolactone is effective against lymphocytic choriomeningitis, eastern equine encephalomyelitis and the MM strain of murine encephalomyelitis viruses contained in plasma or whole blood. LoGrippo *et al.* (1955) reported that β-propiolactone was active against a variety of bacteria and fungi suspended in

[1] Presented at the 57th General Meeting of the Society of American Bacteriologists, Detroit, Michigan, April 28 to May 2, 1957.

phosphate buffer solution and against viruses suspended in various media. Among the microorganisms tested were *Escherichia coli*, *Bacillus proteus*, *Staphylococcus aureus*, spores of *Bacillus globigii*, *Aspergillus niger*, *Microsporum audouini*, *Trichophython mentagrophytes*, Coxsackie (GP-A) virus, poliomyelitis (type II) virus, and rabies (CVS) virus. These same authors and Szilagyi *et al.* (1954) successfully used dilute solutions of β-propiolactone to sterilize human arterial homografts.

The fact that aqueous solutions of β-propiolactone actually were effective against spores as well as other microbiological species, together with its volatility as an organic liquid, led to investigations in this laboratory on its use as a vapor-phase decontaminant.

Experimental Methods

Preparation of Test Microorganisms and Samples

The preliminary evaluation of the activity of β-propiolactone against microorganisms was performed by determining the death rates of selected organisms contained on cloth patches exposed to the vapor of the disinfectant under a variety of environmental conditions.

The test organisms were *Bacillus subtilis* var. *niger* spores (*Bacillus globigii*) and *Micrococcus pyogenes* var. *aureus*. *B. subtilis* var. *niger* spores were produced by growing the organism in liquid casein acid digest media. The organisms were harvested by centrifugation, suspended in water and heat-shocked at 60 C for 30 min. The resulting stock suspension was stored at 4 C; portions of this stock were used for each experiment. *M. pyogenes* var. *aureus* cells were grown on trypticase soy agar slants for 18 to 24 hr at 37 C. The organisms were washed off the slant with water, centrifuged and resuspended in water. A fresh culture was used for each experiment.

β-Propiolactone was evaluated by determining its activity against the microorganisms contained on cotton herringbone twill cloth patches. The patches were contaminated by one of two procedures, depending upon the microorganism involved. Patches were contaminated with *B. subtilis* var. *niger* spores to the extent of 2 to 7 × 10^4 organisms per cm^2 of material by exposure to the fall-out from an aerosol of the test organism. Patches were contaminated with *M. pyogenes* var. *aureus* cells by the direct application of a drop of an aqueous suspension of the test organism. After drying at 25 C, approximately 1 to 5 × 10^4 *M. pyogenes* var. *aureus* organisms could be recovered from a patch.

TABLE 1

Rate of hydrolysis of β-propiolactone

Temperature	Half-Life
C	*min*
10	1080
25	210
50	20
75	5

The major emphasis in these studies was directed toward the evaluation of β-propiolactone against the spores of *B. subtilis* var. *niger*. It is reasonable to presume that any measure of the activity of β-propiolactone against these more resistant types would be at least as great, or greater, when applied to the more sensitive vegetative organisms.

Prior to exposure to β-propiolactone vapor, the cloth patches containing the spores of *B. subtilis* var. *niger* were conditioned for 24 hr to approximately the same relative humidity as that used in the test. This conditioning was accomplished by placing the contaminated patches above a saturated solution of a suitable inorganic salt contained in a desiccator.

Exposure to Decontaminant Vapor

A chamber apparatus in which the temperature and relative humidity could be controlled was used in these studies. The β-propiolactone was aerosolized by means of a "Chicago Atomizer" (Rosebury, 1947) after adjusting the relative humidity and temperature within the chamber to the desired conditions for the test. The contaminated cloth patches suspended on pins were placed in the lactone atmosphere. Patches were removed periodically and placed in dilution blanks containing 0.5 per cent sodium thiosulfate (to neutralize any β-propiolactone carried over on the patch) and 0.1 per cent nonionic detergent (to aid the removal of organisms from the cloth).

The samples were shaken and aliquots plated on nutrient agar enriched with yeast extract. The plates were incubated at 37 C for 48 hr prior to counting.

Analysis

The concentration of β-propiolactone in air was determined by a method developed by B. F. Goodrich Company under contract with the Chemical Corps. The procedure consists of drawing a measured volume of air through Vigreaux[2] bubblers containing 5 ml of alkaline hydroxylamine hydrochloride solution. This solution consists of a 1:1 mixture of 20 per cent, weight-volume, aqueous sodium hydroxide and 20 per cent, weight-volume, aqueous hydroxylamine hydrochloride. β-Propiolactone reacts quantitatively with the hydroxylamine to form a hydroxamic acid. Three ml of acidified ferric chloride containing 10 per cent, weight-volume, ferric chloride dissolved in 6 N hydrochloric acid is added and a colored ferric complex results. The optical density is then determined spectrophotometrically at 500 mμ and converted to

[2] Fisher Scientific Co., Silver Spring, Maryland.

mg per L of air from a standard curve prepared from known concentrations of β-propiolactone.

Effect of concentration, temperature, and humidity on activity of β-propiolactone. As shown in figures 1, 2, and 3, the activity of β-propiolactone is dependent upon the concentration of the chemical vapor, and the relative humidity and temperature of the atmosphere.

At a constant temperature and relative humidity, the time required to kill a specified percentage of spores, that is, 90 per cent, increases as the concentration of lactone is decreased.

As seen in figure 1, the data plotted on a semilog graph yield straight lines. The slope of these lines is a measure of the death rate, k. As pointed out by Phillips (1949) the reciprocal of this death rate, $1/k$, is equal to the time required to kill 90 per cent of the test organisms. Thus, for easy comparison, results are reported in terms of the time required to kill 90 per cent of the test organism (t_{90}). Under the conditions defined in the experiments, a 2-min exposure to a concentration of 1.6 mg of lactone per L or air was required; when a concentration of 0.1 mg of lactone per L or air was used, a 42-min exposure was required to kill 90 per cent of the spores.

The effect of relative humidity on the antimicrobial activity of β-propiolactone is shown in figure 2. A high relative humidity (75 per cent or higher) is required for rapid antibacterial activity. The activity is much less at 60 per cent than at 75 per cent and little activity is noted at a relative humidity of 50 per cent or less. A constant death rate, as indicated by a straight line plot, was obtained only at the higher relative humidities, while curved plots were obtained at the lower relative humidities.

Figure 3 shows the effect of temperature upon the antimicrobial activity of the lactone when concentration and relative humidity were held constant at 1.5 mg of β-propiolactone per L of air and 80 per cent relative humidity. Straight line plots were obtained for the death rate at each temperature. The temperature coefficient (Q_{10}) calculated from results of these experiments was found to be 2 to 3 for each 10 C change in temperature between the range of −10 C to +25 C.

Effect on material. With the concentrations and exposure times recommended, β-propiolactone vapor

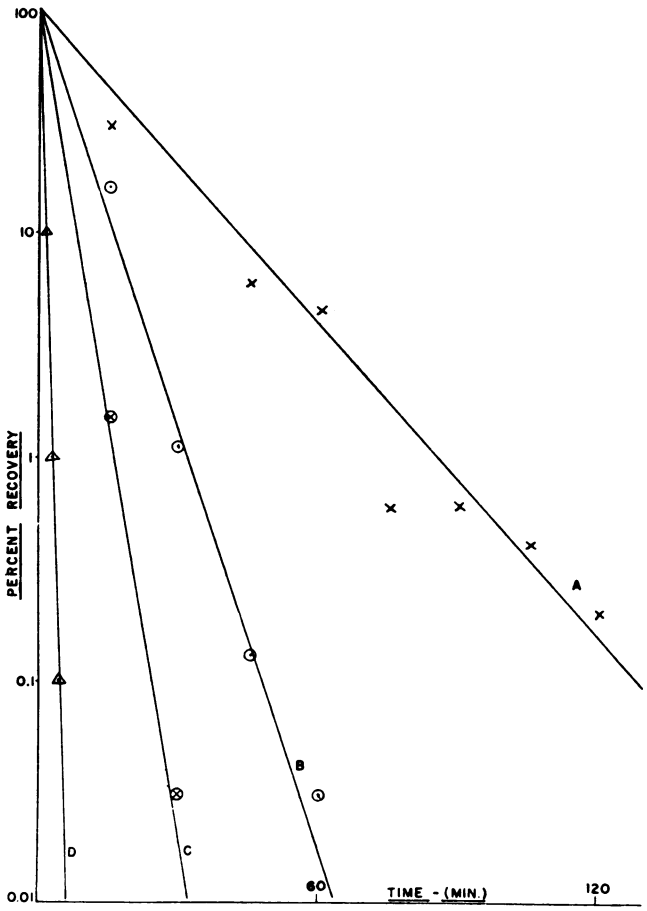


Figure 1. Effect of β-propiolactone concentration on death rate of spores of *Bacillus subtilis* var. *niger*. Relative humidity, 80 ± 5 per cent; temperature, 27 ± 2 C. Concentrations (mg per L): *A*, 0.1; *B*, 0.2; *C*, 0.4; *D*, 1.6.

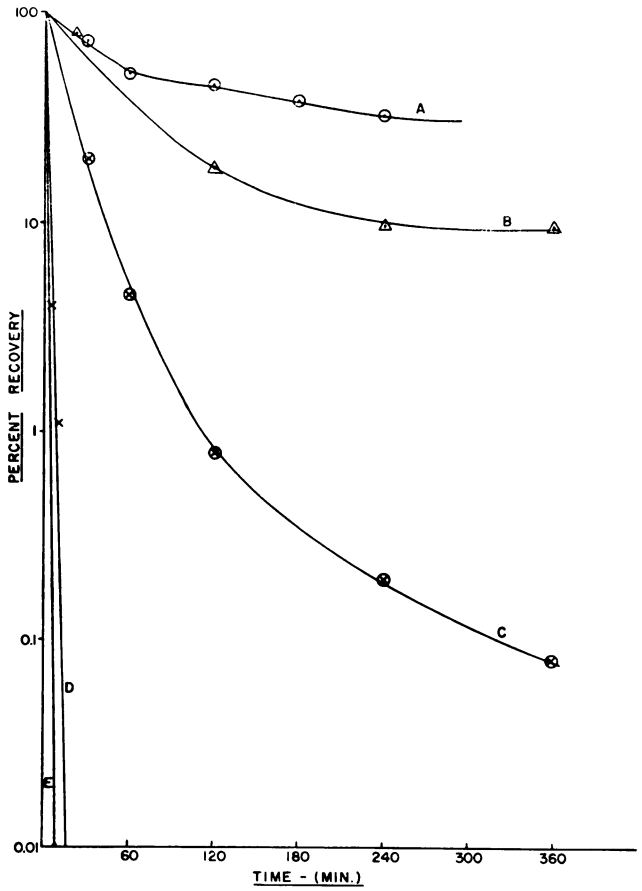


Figure 2. Effect of relative humidity on death rate of spores of *Bacillus subtilis* var. *niger* exposed to β-propiolactone vapor. Lactone concentration, 1.5 ± 0.3 mg per L; temperature, 27 ± 2 C. Relative humidities: *A*, 45 per cent; *B*, 50 per cent; *C*, 60 per cent; *D*, 75 per cent; *E*, 85 per cent.

does not appear to corrode metals or have a deleterious effect on materials. Sensitive laboratory equipment which could be adversely affected by extremely small amounts of corrosion have not been tested to date.

Flammability. β-Propiolactone is not flammable in air in concentrations which theoretically can exist under normal atmospheric conditions. It has been estimated that 2 per cent by volume of β-propiolactone in air would produce a flammable mixture. The vapor pressure of β-propiolactone is such that at 30 C the concentration in air at the saturation level is only about 0.6 per cent by volume, hence no flammable hazard can exist; this concentration is considerably above the concentration of disinfectant necessary for sterilization of enclosed spaces.

Toxicity. Available information on the toxicity of β-propiolactone is somewhat limited. It has been determined by human volunteer experiments that humans can detect the odor of a concentration of 0.05 mg of lactone per L of air; because of its lachrymatory properties, human beings cannot tolerate concentrations greater than 0.1 mg per L of air for longer than 5 min. Undiluted β-propiolactone produces no harmful effect on skin if washed off immediately; however, if it is held in contact with the skin for a period in excess of one-half hr, blisters are formed.

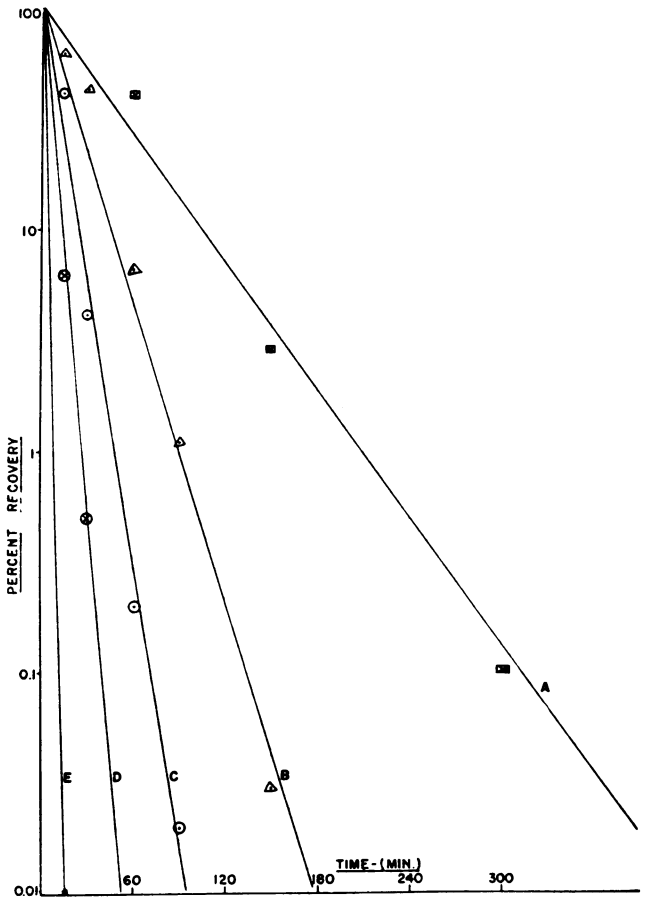


Figure 3. Effect of temperature on death rate of spores of *Bacillus subtilis* var. *niger* exposed to β-propiolactone vapor. Lactone concentration, 1.5 ± 0.3 mg per L; relative humidity, 80 ± 5 per cent. Temperatures: *A*, −10 C; *B*, −2 C; *C*, 6 C; *D*, 15 C; *E*, 25 C.

Applications. β-Propiolactone has been employed successfully to decontaminate chambers up to 3000 cubic feet and buildings up to 75,000 cubic feet in volume. A summary of one such test is given below.

A two-story building with a volume of 50,000 cubic feet was contaminated at various locations with *B. subtilis* var. *niger* spores. The interior building temperature was maintained at 24 C, and water vapor was introduced to raise the relative humidity to 80 per cent. Sixteen L of undiluted β-propiolactone were disseminated in the building as an aerosol by means of a Todd Insecticidal Fog Applicator[3] placed at the front entrance of the building. An average concentration of about 5 mg of β-propiolactone per L of air was maintained throughout the period of treatment. After a 2-hr exposure to the disinfectant, no viable spores were recovered from a total of 55 biological samples. The building was habitable after 2 days' normal aeration, that is, with doors and windows opened. No damage to painted surfaces or metal fixtures in the building was noted.

Discussion

Preliminary data on the relative resistances of *M. pyogenes* var. *aureus* and *B. subtilis* var. *niger* spores showed that under similar conditions of temperature and relative humidity of the atmosphere, and concentration of the lactone, the spores were only 4 to 5 times more resistant to the disinfectant than were *M. pyogenes* var. *aureus* cells. LoGrippo *et al.* (1955) found the same ratio of resistance between spores and vegetative cells upon exposure to aqueous solution of β-propiolactone.

Based on this similarity in rate of inactivation of spores and nonsporeforming organisms and the chemical reactions of lactones, β-propiolactone presumably acts as an alkylating agent, the lactone ring splitting at either the first or third carbon atom. This mechanism of disinfectant action contrasts significantly with other modes of action, such as those involving heat-inactivation, heavy metals, or oxidation, in which appreciable difference between the activity against vegetative cells and spores is noted (Phillips, 1952).

Data collected in this laboratory show that β-propiolactone vapor when employed under conditions of maximum effectiveness is approximately 25 times more active as a vapor phase disinfectant than formaldehyde, approximately 4000 times more active than ethylene oxide and 50,000 times more active

[3] Combustion Equipment Division, Todd Shipyards Corp., Elmhurst, Long Island, New York.

than methyl bromide. These results are based on the Ct_{90} values obtained at the relative humidity of greatest activity for each of the disinfectants. (Ct_{90} = The concentration of the disinfectant vapor in mg per L of air times min required to kill 90 per cent of the spores.)

The fact that β-propiolactone is many times more active than ethylene oxide does not imply that the lactone can be substituted for the oxide in all situations. Ethylene oxide possesses several very desirable properties which are lacking in β-propiolactone. Two of these properties are (1) high degree of penetration and (2) effectiveness at low relative humidities. These properties make ethylene oxide an excellent decontaminant for clothing, bedding, books, drugs, delicate equipment, and numerous other items. It is suggested that β-propiolactone may be used as a replacement for formaldehyde in gaseous decontamination. The lactone does not possess some of the undesirable characteristics of formaldehyde, specifically its persistence. As pointed out by Phillips, formaldehyde upon spraying, condenses on surfaces and polymerizes. As a result it is extremely difficult to rid the surface of the polymer. The polymer, paraformaldehyde, volatilizes slowly making it necessary to aerate enclosures that have been decontaminated with formaldehyde as long as a week or more before the enclosure is habitable. β-Propiolactone does not condense as a nonvolatile residue and thus the time required to aerate an enclosure after application of the disinfectant is considerably less than that for formaldehyde. A day or two (with normal aeration) or less (with forced ventilation) is sufficient to eliminate any objectionable concentrations of β-propiolactone.

As shown by data in figure 3, β-propiolactone is active even at relatively low temperatures. Ethylene oxide and formaldehyde, on the other hand, demonstrate very low sporicidal activity at temperatures of 10 C or below. Thus the latter two vapor disinfectants are limited to use at room temperature or above but the lactone can be used at lower temperatures.

ACKNOWLEDGMENTS

The work reported in this article represents the combined effort of many individuals associated with these laboratories. Therefore, without attempting to assign specific credit for each contribution, the authors wish to express their appreciation to all those individuals who participated in these studies and in the preparation of this manuscript.

SUMMARY

Data are presented which show that the rate at which spores are inactivated by β-propiolactone vapor is directly dependent upon the concentration of the chemical and upon the relative humidity and temperature of the atmosphere.

β-Propiolactone, when used properly, is a very effective vapor-phase disinfectant for the decontamination of chambers, rooms, and buildings.

This disinfectant is not recommended as a substitute for ethylene oxide since it lacks the penetrating power of ethylene oxide and furthermore requires a high moisture content for its activity. Instead, because of its greater activity and ease of removal, it is suggested as a replacement for formaldehyde. Preliminary observations indicate that lactone can be used as a vapor disinfectant in almost any situation in which formaldehyde currently is used.

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