Method for Disinfecting Large Enclosures with β-Propiolactone Vapor

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This paper presents a practical method for disinfecting large enclosures with β-propiolactone (BPL) vapor, as well as precautions to be taken when using the chemical. Included are the summaries of tests in which a U. S. Army barrack, a hospital ward dayroom, an entire hospital wing, and a wing of an animal housing building were treated by the described procedure.

The application of liquid disinfectants to decontaminate large enclosed areas, such as warehouses, hospital wards, biological and pharmaceutical laboratories, and animal quarters, is not only tedious and uncertain because of the expanse of the surfaces, but frequently results in corrosion or other damage. The ideal method of decontaminating such areas is by the use of a bactericidal gas which will permeate the enclosure and sterilize all surfaces as well as the immediate air. The gaseous sterilant should not be so penetrating that it cannot be contained long enough to sterilize the enclosure, nor should it produce deposits or residues which require removal. In addition, the agent should not be corrosive at the concentration recommended for use.

The chemicals which have been used as gaseous disinfectants in the past fall short of the limitations set forth above. For example, chlorine and sulfur dioxide are too corrosive; formaldehyde tends to deposit as a solid polymer which slowly reverts to the irritating gaseous form, thus posing a problem of aeration following use; and ethylene oxide gas is so penetrating that it escapes all but tightly sealed containers, such as autoclaves. β-Propiolactone[1] vapor on the other hand appears to meet more closely the above criteria and thus is a more suitable gaseous disinfectant for rooms or buildings (Hoffman and Warshowsky, 1958).

β-Propiolactone is a colorless liquid at room temperature. It boils at 162 C, has a specific gravity of 1.1490, and a vapor pressure of about 3.4 mm Hg at 25 C. The commercial grade lactone (97 per cent BPL) is recommended for use in building decontamination because it is considerably cheaper than the purified grade. In use concentration the vapor is nonflammable and noncorrosive. However BPL is toxic to man. This property is discussed under the section entitled "Precautions."

[1] Celanese Corporation of America, New York, New York.

BPL vapor was shown in this laboratory to have sporicidal, bactericidal, rickettsicidal, and virucidal properties. Hoffman and Warshowsky (1958) reported that BPL vapor effectively inactivates *Bacillus subtilis* var. *niger* spores and *Staphylococcus aureus* cells. Dawson *et al.* (1959, 1960) found BPL vapor to inactivate the causative agents of Venezuelan equine encephalomyelitis, smallpox, yellow fever, psittacosis, and Q fever. Unpublished studies in the authors' laboratory demonstrate that the chemical vapor is also effective against *Bacillus anthracis* spores, *Clostridium sporogenes* spores, and *Mycobacterium tuberculosis*.

General Method for Disinfecting Enclosed Spaces with BPL Vapor

The following procedure is applicable to the disinfection of chambers, rooms, and large buildings.

Preparation of the structure. It is not necessary that an enclosure be airtight to decontaminate the interior with BPL; however it is advisable to seal large cracks with masking tape to prevent loss of the chemical. Naturally all air conditioners and ventilating systems which might extract the vapor should be shut off and preferably sealed. If only one room or a part of a building is to be treated, special care should be taken to seal crevices through which the chemical might seep into adjoining rooms where an intolerable concentration might ensue. The room (or rooms) should have a separate source of ventilation such as an outside window or door which can be opened for aeration purposes following the lactone treatment.

All cabinets, drawers, and inside doors should be opened to facilitate contact with the disinfectant. Moreover, it is recommended that electric fans be placed in critical locations to assure circulation of the BPL vapor down corridors, into side rooms, and even under furniture.

Temperature and relative humidity. The bactericidal activity of BPL vapor is a direct function of the concentration, relative humidity, and temperature at which it is used. For optimal activity, it is recommended that the temperature be maintained above 24 C (75 F) and the relative humidity be kept at 70 per cent or higher. The effectiveness of this disinfectant decreases rapidly as the relative humidity drops below 70 per

cent. As shown by Hoffman and Warshowsky (1958) the relative humidity is much more critical than the temperature in determining the rate of microbial inactivation with BPL.

Most structures are equipped with a heating system by which the temperature can be maintained at 24 C or higher. If necessary, the relative humidity can be elevated by an aerosol of water from a vaporizer or the same device used to disseminate the disinfectant. Once the humidity has been elevated, it is often necessary to continue to spray water at a reduced rate to maintain the high relative humidity. This can be done by the use of one or more small vaporizers. If the room is equipped with an autoclave, both the temperature and the relative humidity may be increased by opening the autoclave door, releasing the steam until the desired conditions are reached, then lowering the steam to maintain these conditions. A recording hygrothermograph is useful for reference at this time and during the exposure.

Dissemination of the lactone. The recommended initial concentration of BPL to decontaminate an enclosure is 2 to 4 mg per L of air. To obtain this concentration it is generally necessary to aerosolize 9 to 12 mg BPL per L of air. The exact amount will depend upon the efficiency of the sprayer and the nature and quantity of adsorptive material present in the room. In other words, a room which has a large quantity of highly adsorptive material such as rugs, drapes, overstuffed furniture, and so forth, would require the dissemination of considerably more of the lactone than a room without these materials. To decontaminate an ordinary laboratory or room, disseminate 1 gallon of BPL for each 16,000 cubic feet of space. To decontaminate rooms having an unusual amount of adsorptive surface, spray 1 gallon of the lactone for each 12,000 cubic feet. The spraying of more than 1 gallon of the lactone per 12,000 cubic feet of space, or the use of an inefficient spray device, can result in the liquid lactone condensing on some surfaces.

Almost any commercial insecticide sprayer may be used to disseminate the BPL. Two requirements are placed on the selection of a spray device. First, it must disseminate the chemical in sufficiently small particle size to prevent falling out or "raining" on surfaces; and second, if the device is a thermal type generator, the chemical should not be subjected to a temperature high enough to cause decomposition. If the spray device has selected spray rate settings, it is usually advisable to spray at the finest setting and thus spray a longer time. If the sprayers are electrically powered, they may be filled, placed in the room, and controlled with an outside master switch. One or more smaller portable atomizers are convenient for the treatment of rooms.

At the recommended 2 to 4 mg of BPL per L of air and 2 hr contact time, no damage to material by the lactone vapor has been observed. However, care should be taken when spraying. Liquid lactone can collect on surfaces if the sprayer disseminates too large a particle or if the sprayer is positioned too close to surfaces. Extended contact with the liquid can have two effects on the surfaces: (a) it may leave a residue that is difficult to remove and (b) it can dissolve or craze certain finishes (wax, varnish, and some paints) or plastics. If an excess of the liquid should deposit on flooring near the sprayer, it can usually be washed off without damage, although rewaxing will probably be necessary.

Contact time and subsequent aeration. The recommended contact time for sterilization by this procedure is 2 hr. At the end of the 2-hr period, doors and windows should be opened to facilitate aeration of the enclosure. If only the upper story of a building is treated, the windows of the floor beneath should be closed to prevent entry of the vapor. If it is necessary to enter the enclosure to open the windows, a gas mask with a charcoal canister must be worn, since the concentration of BPL will still be above that which can be tolerated by man. Forced ventilation with exhaust fans will shorten the aeration period. The treated area should be aerated until the lactone odor has disappeared or is unobjectionable.

Quantitative analysis of BPL. If desirable, the BPL vapor concentration can be determined during the exposure by withdrawing air samples and analyzing them spectrometrically (Hoffman and Warshowsky, 1958).

Precautions

Toxicity. The toxicity of BPL for animals and man was determined by Ben Venue Laboratories (Unpublished Data). These studies revealed that 40 per cent mortality of rats resulted after a 2-hr exposure to an average concentration of 0.36 mg BPL per L of air. A 2-hr exposure to an average concentration of 0.72 mg BPL per L of air resulted in 80 per cent mortality of rats.

Ben Venue Laboratories also determined the detectable and tolerable concentration of BPL vapor. It was found that the lowest detectable concentration for man was 0.05 mg per L of air, and that a concentration as low as 0.1 mg per L of air was intolerable for longer than 5 min. Thus, the vapor is highly irritating at concentrations not much above its detectable limit, and at concentrations below its acute toxic level, so that BPL, like formaldehyde, serves as its own warning device. Areas which personnel can enter unmasked without discomfort are no longer considered toxic.[2]

[2] It was recently suggested that a chemical test rather than absence of residual odor be used to determine when the BPL concentration is reduced to a safe level. The gas detection tubes developed by the Chemical Corps, and recently incorporated

BPL can also act upon the skin. Extended contact with the liquid (Ben Venue Laboratories, Unpublished Data) or the vapor (Feazel and Lang, 1959) can cause erythema and vesication. Skin irritation has most often been noticed where the vapor was adsorbed by sweaty clothing worn against the skin. Undiluted BPL produces no harmful effect on the skin if promptly washed off with water.

The carcinogenic effect of BPL has been the subject of considerable investigation. Walpole *et al.* (1954) showed that frequent subcutaneous injections of dilute BPL were carcinogenic for rat tissue. Roe and Salamon (1955) and Roe and Glendenning (1957) found that tumors appeared in mice after 27 weekly applications of subulcerative doses of BPL to their skin. Tumors not only appeared sooner but were sometimes malignant if ulcerative doses of BPL were repeatedly applied to mouse skin. These data indicate that repeated exposure to subirritating doses of BPL vapor should be avoided.

Personnel should be masked if it is necessary for them to enter an area containing an irritative level of BPL. If they intend to remain in the area for more than a few minutes, gas-impermeable protective clothing should be worn. When entering areas for only a few minutes to remove equipment or to open windows for ventilation, the following precautions should be taken:

Wear a gas mask equipped with a charcoal canister.

Wear washable outer clothing, such as laboratory overalls. Tie these at wrists and ankles to prevent vapor entering these areas through bellows action as one moves about.

Do not remain in the BPL atmosphere with this type clothing for more than a few minutes.

Remove the outer clothing immediately after emerging from the lactone atmosphere.

It is always advisable to shower after exposure to BPL vapor and to don clean clothing as soon as possible.

Residual. A fine film may deposit on surfaces if an excess aerosol of BPL is used, or if the spray device is inefficient. This film is the result of "fallout" and is not encountered under the proper conditions of dissemination.

Another type of surface film, an oily residue, has been reported in a few instances following the use of BPL. Experiments in our laboratory indicate that a residue that is difficult to remove is produced by spraying BPL of lower purity than that specified (97 per cent) by the manufacturer of the chemical. It was determined that BPL of 87 per cent purity, which can have a clear watery appearance, produces a noticeable residue on surfaces after dissemination. The impurity, which is water insoluble, is probably a BPL polymer. BPL containing less impurity produces less of the residue, whereas 97 per cent pure material produces no residue when properly disseminated. The formation of polymer in BPL may be retarded by storing the chemical in chemically clean containers and at refrigeration temperature.

Disinfection of a Two-Story Army Barrack, Dayroom, a Hospital Ward, an Entire Hospital Wing, and a Wing of an Animal Housing Building

The method, described above, for disinfecting large enclosures with BPL vapor, has been used by our personnel to decontaminate laboratories, barracks, hospitals, and animal rooms. Summaries of a few of these tests are presented below.

Disinfection of a two-story army barrack. A two-story army barrack with a volume of 50,000 cubic feet was treated with BPL vapor. Numerous surfaces within the building were first contaminated with spores of *Bacillus subtilis* var. *niger* (*B. globigii*). The relative humidity was raised to 80 per cent by spraying water and the building temperature was maintained at 24 C. The BPL was sprayed into the barrack by means of a Todd Insecticidal Fog Applicator.[3] After a 2-hr exposure to the disinfectant, 55 biological samples were taken from which no viable spores were recovered. The building could be occupied after 1 to 2 days of normal aeration, that is with the windows and doors open. No damage to painted surfaces or metal fixtures was noted.

Disinfection of a furnished hospital dayroom. The hospital ward dayroom treated in these tests did not lend itself to the normal means of sterilization (application of liquid disinfectants) because of wall to wall carpeting, drapes, overstuffed furniture, books, and a television set. Two tests were conducted. The first was carried out on a cold day which necessitated using radiators to maintain a temperature of 24 C and spraying water to raise the relative humidity. Patches contaminated with known numbers of spores of *B. subtilis* var. *niger* were used as an indicator of the success of the decontamination procedure. The number of viable spores was greatly reduced; however only 50 per cent of these patches were sterile after exposure to the BPL vapor. Two evident reasons for the incomplete sterilization were the low BPL concentration and low moisture content in the air. Both dropped rapidly after being introduced into the room, because of condensation on the cold outside walls and windows and adsorption by the drapes, rugs, and so forth. The room was treated the second time on a warm and humid day,

in Civil Defense survival kits, will respond to BPL concentrations lower than 0.01 mg per L. This test is based on the blue color caused by the alkylation of gamma (*p*-nitrobenzyl) pyridine adsorbed on silica gel inside a small glass tube through which air is drawn by a hand pump. The reaction is discussed in Analytical Chemistry, **27**, 1435–1439 (1955) and the kit is described in Chemical Corps Purchase Description 197-54-627 and Chemical Corps Drawing D5-77-206.

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

thus eliminating the need of elevating the temperature and relative humidity. An aerosol of BPL was made by means of two Tokheim Power Atomizers.[4] Fans, placed at various locations within the room, were run throughout the exposure period to insure a thorough distribution of the disinfectant. As a check on the decontamination procedure, 12 patches contaminated with spores of *B. subtilis* var. *niger* were placed at various sites throughout the room before spraying. All but one of the test patches were found to be sterile after exposure to BPL. The one unsterile patch was obtained from a site under the rug and even at that, the viable count was reduced 99.999 per cent. Approximately 1 hr after opening the windows to aerate, unmasked personnel could enter the room. No damage, due to the BPL treatment, to any material or items in the room was noted.

Disinfection of an army hospital ward (Woodward and Clark, 1960). One wing of a one-story army hospital with a volume of 89,000 cubic feet was treated with BPL vapor. The wing contained bedrooms, offices, kitchen, operating room, preparation room, X-ray room, and a dayroom. As in the previous tests, spores of *B. subtilis* var. *niger* were used as an index of the success of the decontamination procedure. Thirty-one sites throughout the ward were contaminated with bacterial spores before spraying the BPL. The temperature and relative humidity averaged 26 C and 87 per cent. Following a 2-hr exposure to the disinfectant only 1 of the 31 biological sampling sites was not sterile; however, even at this site the contamination level was reduced more than 99.99 per cent. Several varnished tables and toilet seats were damaged by liquid BPL. The dispenser was not operating correctly thus permitting the liquid BPL to collect on some surfaces. No damage was noted to other items present at the time of disinfection. Among these items were an X-ray machine, surgical equipment, resuscitator, beds, drapes, furniture, air conditioners, and a television set. One hr after opening the doors and windows following the BPL treatment the ward could be entered with only slight irritation due to residual BPL. Twenty-four hr later the ward was available for occupancy. The results of this work are reported in more detail by Woodward and Clark (1960).

Decontamination of an animal housing room. At the request of the pharmaceutical industry, a demonstration of the procedure to decontaminate large enclosed areas by the use of BPL vapor was staged at Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania. For this purpose, a large room of an animal housing building was treated with BPL. Following the treatment only 1 of 16 previously contaminated patches was not sterile; however even at that, the bacterial spore count was reduced three orders of magnitude on the patch. With the aid of exhaust fans located along two walls, the building was aerated rapidly. Twenty min after opening the doors and starting the fans, the structure could be entered without a noticeable irritation of the respiratory tract due to residual BPL vapor.

[4] Tokheim, Fort Wayne, Indiana.

Summary

The general procedure for decontaminating enclosed spaces with β-propiolactone (BPL) is relatively simple. In preparing an enclosure, large cracks around doors, windows, and so forth should be sealed with masking tape. Ventilating systems must be shut off and preferably sealed. For optimal activity of the disinfectant, the relative humidity should be 70 per cent or higher, and the temperature preferably kept at 24 C or higher. Most commercial insecticide sprayers can be used to disseminate BPL. One gallon of the chemical should be sprayed for each 12,000 to 16,000 cubic feet of space. Generally, a 2-hr contact time is sufficient for decontamination. Under normal conditions of aeration, a day is sufficient before the area can be occupied, although the time for aeration can be greatly reduced by using forced ventilation. Because of the toxicity of BPL care must be taken when handling the chemical. BPL of the purity specified by the manufacturer (97 per cent) does not produce a residue upon spraying; however BPL containing polymers of the chemical will cause the formation of a residue which is difficult to remove. Summaries of decontamination trials presented indicate the effectiveness of the described decontamination procedure.

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