

Effect of Relative Humidity on Formaldehyde Decontamination

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Death rate studies were conducted to determine the effect of varying the concentration, humidity, and type of surface on the sporicidal activity of formaldehyde gas. Washed and unwashed spores were similarly exposed to detect the influence of residual nutrient growth medium upon the rate of kill. The results indicated that the sporicidal activity of formaldehyde gas varies directly with its concentration. Relative humidities (RH) over 50% proved essential for sterility. Spores on a porous surface (cotton cloth) were more readily killed at lower RH than those on a nonporous surface (glass). The reverse occurred at very high RH. At 75% RH, the unwashed spores on glass were killed faster than the washed spores.

In the past few years, interest has been renewed in the use of formaldehyde gas for building and room decontamination. Two factors that have encouraged this interest are the Vineland (7) system of using paraformaldehyde as the formaldehyde source and the current inability to procure beta-propiolactone for decontamination purposes. One technique of using paraformaldehyde in the treatment of large enclosures was described by Taylor et al. (6). As part of a study on the effectiveness of formaldehyde as a decontaminant, two studies on relative humidity (RH) have been performed in this laboratory. Braswell et al. (1) reported on the effect of RH on the adsorption and Hoffman and Spiner (3) on the effect of RH on the penetrability of formaldehyde gas. However, the effect of RH on the biological activity was not adequately investigated. Nordgren (5) reported that the gas has some, although low, sporicidal activity even at 2.5% RH. The activity was found to increase as the RH increased to 50%, but there was little increase at higher RH. The Committee on Formaldehyde Disinfection of the Public Health Laboratory Service of Great Britain (2) confirmed the latter point in general and concluded that, even though the optimum activity of formaldehyde occurs at 80 to 90% RH, the increase in activity between 58 and 100% RH is not significant. Neither of these studies utilized formaldehyde concentrations currently recommended for decontamination purposes, nor did they compare its activity against spores on porous and nonporous surfaces. These factors were investigated and are reported in this study.

MATERIALS AND METHODS

Preparation of samples and microorganisms. The effect of RH on the sporicidal activity of formaldehyde gas was determined against *Bacillus subtilis* var. *niger* spores on cotton cloth and glass surfaces. These surfaces were selected as representative of absorptive and nonabsorptive surfaces. Cotton cloth patches [0.5 inch (1.27 cm) diameter] and 0.5-inch-square pieces of glass (cut from hard, noncorrosive microscope slides) were contaminated with 0.05 ml of aqueous spore suspensions containing 40 to 100 million viable spores per milliliter. The patches were transferred to large desiccators containing saturated salt solutions or pure water to maintain an RH of 11 to 100% at 25 C. The saturated salt solutions used to maintain humidities of 11, 33, 53, and 75% were lithium chloride, magnesium chloride, nickel chloride, and sodium chloride, respectively. Water was used for 100% RH. The contaminated patches were preconditioned to the desired RH for a minimum of 3 days before being exposed to formaldehyde.

Exposure to formaldehyde vapor. The exposure to formaldehyde was performed at the same RH as that to which the surfaces were preconditioned. The technique was the same as that described by Hoffman and Spiner (3) which involved rapidly transferring two cloth patches and two glass squares from a preconditioning desiccator to another desiccator (9.4 liters) equipped with special side arms on the top (Fig. 1). Then the RH was quickly adjusted by passing air of the same humidity used for preconditioning through the desiccator. The RH of the air was regulated by blending moist and dry air to give the desired humidity. A calculated amount of paraformaldehyde was weighed in a small glass boat and placed in the side arm of the desiccator top. The side arm was closed with a ground-glass stopper and heated gently for 1 to 2 min with a bunsen burner to vaporize the formalde-

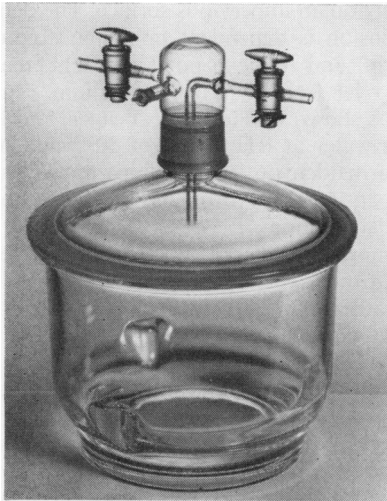


FIG. 1. Formaldehyde exposure chamber.

hyde. The desiccators were then stored for 0.5, 1, 2, 4, 8, or 24 hr at 25 C. Three formaldehyde concentrations were used in this study: 1.1, 3.5, and 10.6 mg/liter. The latter concentration is above saturation but was recommended by Vineland and used by Taylor et al. Each trial was repeated three times by using two glass squares and two cloth patches for each test. After exposure to formaldehyde, the cloth patches and glass squares were placed in individual 10-ml sterile distilled water blanks and shaken thoroughly, and samples were placed in petri dishes. Tryptose agar was used as the nutrient medium. All plates were incubated for 3 to 5 days at 37 C before counting.

RESULTS AND DISCUSSION

The results of this study are shown in Fig. 2 and 3. Figure 2 demonstrates the type of curve obtained when *B. subtilis* var. *niger* spores were exposed to 3.5 mg of formaldehyde per liter of air at various RH. Straight line death rates were obtained on semi-log graph paper for all but the 11% RH curves. At this low RH, a tailing effect is evident for spores on cloth. The same effect was noted for spores on glass, but it occurred only after 8 hr of exposure and consequently is not evident on the graph.

Figure 3 summarizes all of the results obtained in this study, i.e., the decimal reduction times (*D* values) for *B. subtilis* spores exposed to three formaldehyde concentrations, five RH values, and on two surfaces. The checkered portion on top of each bar graph indicates the variation in *D* values obtained in the three trials at each condition. The dotted extensions on the 11% RH bar graphs indicate tailing of the death curve after a straight-line death of one to four orders of magnitude.

The following points of interest are evident

from these results. (i) Elevating the RH from 11 to 53% accelerates the rate of kill by formaldehyde gas. The change is less at higher RH (53 to 100%). This observation agrees with the findings of Nordgren and the Committee on Formalde-

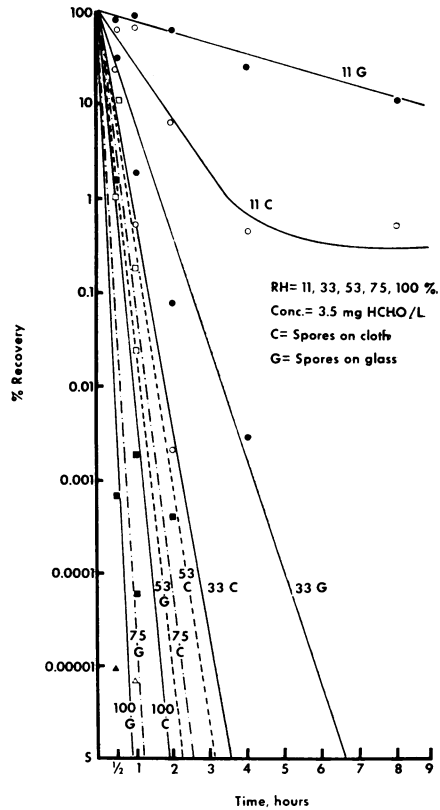


FIG. 2. Death rates of *Bacillus subtilis* var. *niger* spores exposed to formaldehyde gas.

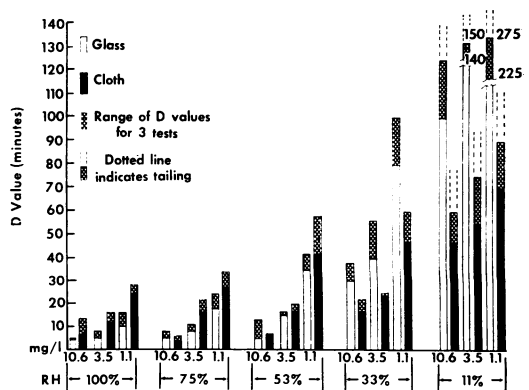


FIG. 3. Decimal reduction (*D*) times for *Bacillus subtilis* var. *niger* spores exposed to formaldehyde gas from paraformaldehyde.

hyde Disinfection. (ii) The higher the formaldehyde concentration the faster the sporicidal activity. As previously mentioned, 10.6 mg/liter is above air saturation; thus, a white precipitate (paraformaldehyde) was often observed on many surfaces within the desiccator 1 hr or so after the initial vaporization of the paraformaldehyde. A more desirable concentration for routine use is probably on the order of 3.5 mg/liter. If, however, the enclosure being treated has much absorptive material such as carpeting, drapes, fabric-covered furniture, etc., a higher initial concentration of formaldehyde is recommended. (iii) At very high humidity, spores on glass were killed more readily than the spores on cloth. At lower RH, the reverse was found. In an attempt to explain this, a drop of the original spore suspension was dried on a glass slide. The dried preparation was then observed under the microscope as the RH of the surrounding atmosphere was increased. At the high RH, the dried particles were observed to absorb water, producing small droplets throughout the previously dried particles. This indicated the presence of extraneous hygroscopic material, possibly organic or inorganic constituents of the growth medium. Another test was run, although the data are not included here, which involved taking a portion of the original suspension and washing it five times in sterile distilled water to remove any extraneous material. This suspension plus the original one were tested concurrently at 75 and 100% RH exposed to 3.5 mg of formaldehyde per liter. The results showed no difference in the rate of kill of clean and non-cleaned spores on the cloth surface at 100% RH. The same was true for clean and noncleaned spores on glass, except that their kill rate was faster than on cloth. At 75% RH, there was no difference in the rate of kill of clean spores on cloth or glass, but the unwashed spores were killed faster on glass.

Thus, one may assume that the degree of cleanliness of a microbial suspension can have a direct bearing on the rate of kill by formaldehyde gas,

especially on an impervious surface. The debris in a suspension is dependent upon the amount of expended and nonexpended nutrient material carried over from the growth medium. Nutrient broth, as shown by Hoffman, Yeager, and Kaye, absorbs water at RH values of 50% and higher (4). Formaldehyde gas is known to dissolve readily in aqueous droplets, giving a rapidly sporicidal solution.

Cleaning the bacterial spores had less effect on the death rate when the spores were exposed on cloth, rather than on glass. The reason for this is probably because water absorbed by the particles at high RH is in turn readily absorbed by the cloth fibers; therefore, a concentrated formaldehyde solution does not exist surrounding the spores. Conversely, on glass the solution remains surrounding the organisms.

It is evident that the RH, gas concentration, degree of microbial cleanliness, and the nature of the surface holding the organism can greatly affect the rate at which formaldehyde gas kills spores. These variables, plus others, make it difficult to assign precise exposure times for large-scale formaldehyde decontamination. It is possible, however, to assign approximate exposures, but these should be based on such factors as the RH, chemical concentration, temperature, and degree of microbial contamination.

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