Effect of Relative Humidity on the Penetrability and Sporicidal Activity of Formaldehyde

ROBERT K. HOFFMAN AND DAVID R. SPINNER
Department of the Army, Fort Detrick, Frederick, Maryland 21701

Received for publication 13 July 1970

The effect of relative humidity (RH) on formaldehyde penetration of paper, glassine, and cotton was determined by the death rate of bacterial spores in glass tubes covered with these materials. The data show that paper is readily penetrated regardless of RH, but the RH greatly affects the penetration rate of glassine and cotton. A comparison was also made of the effect of RH on the penetrability of formaldehyde generated from Formalin and paraformaldehyde. At low RH, all three closures were penetrated more readily by formaldehyde from paraformaldehyde than from Formalin, but no difference in the two was observed at high RH. It is felt that the difference at low RH is primarily due to the condensation of the vaporized Formalin.

From the limited information available, it appears that formaldehyde vapor is not an effective means of sterilizing microorganisms embedded in any type of material. Phillips (2), for instance, stated that formaldehyde at high relative humidity (RH) would sterilize surfaces in a room but that it was "difficult to sterilize surfaces covered in any manner or to sterilize throughout porous materials." Sykes (3) stated that the use of formaldehyde as a sterilizing vapor was limited because of its lack of penetration, and therefore other means, such as vacuum, must be employed to assist the penetration to sterilize blankets and mattresses. The Committee on Formaldehyde Disinfection (1) concluded from their studies that the chemical vapor should be used only when no other method is available. They recommended that it not be used for disinfection of fabric contaminated with smallpox virus or bacterial spores. The conclusions of the Committee were based on studies that depended on the penetrability of formaldehyde through 3 to 15 layers of blanket. Their tests were performed over Formalin solution, an atmosphere that would have a high RH. None of these investigators studied the effect of RH on formaldehyde penetrability. The present study was undertaken for the express purpose of investigating this factor.

MATERIALS AND METHODS

Preparation of test microorganisms and samples. The effect of RH on the penetrability of formaldehyde gas through tablet paper, glassine paper, and cotton was determined by the death rates of bacterial spores within glass test tubes using the three materials for closure (Fig. 1). The test organisms were Bacillus subtilis var niger spores. Pieces of Whatman no. 42 filter paper (5/8 by 5/8 inch; ca. .95 by .95 cm) were contaminated with one measured drop of the aqueous spore suspension containing 10 to 20 million viable spores per ml. The patches were transferred to individual glass tubes (.5 by 6.0 inches; ca. 1.27 by 15.5 cm) and located about half way down the tube. The tubes were closed by placing a piece of white tablet paper (1.25 by 1.25 inches; ca. 3.17 by 3.17 cm) or Lily no. 4 glassine powder paper over the mouth and taping tightly around the sides with two layers of 1-inch masking tape. Other tubes were closed with a 1-inch tight plug of absorbent cotton. The tubes were then laid on racks in 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 in the test tubes were preconditioned to the desired RH for a minimum of 3 days before being exposed to formaldehyde.

Exposure to formaldehyde vapor. All formaldehyde exposures were performed at the same RH as that to which the patches were preconditioned. Three tubes, one with each closure, from a specific preconditioning RH were placed in another desiccator (9.4 liters) equipped with special side arms on the top (Fig. 2), and the RH was rapidly adjusted by passing air of that RH through the desiccator at 10 to 15 liters/min for 10 to 15 min. However, when Formalin, which contains 50% water, was used, the desiccator was equilibrated to a lower RH to compensate for the
additional water. The RH of the air entering the desiccator was regulated by blending moist and dry air to give the desired humidity, as measured by a humidity sensing element (Hydrodynamics, Inc., Silver Spring Md.). The desired amount of paraformaldehyde or Formalin was weighed in a small glass boat and then placed in the side arm of the desiccator top. The side arm was closed by a ground-glass stopper, the stopcock leading to the glass extension tube in the desiccator top was opened to allow for pressure release, and the side arm was heated gently with a bunsen burner to vaporize the formaldehyde. The entire glass top was also heated to prevent formaldehyde condensation on it; however, the rest of the desiccator was not

![Image 1](image1.png)

**FIG. 1.** Exposure tubes with paper, glassine, and cotton plug closures.

![Image 2](image2.png)

**FIG. 2.** Formaldehyde exposure chamber with modified top.

**TABLE 1.** Per cent recovery of B. subtilis var niger spores in test tubes with various closures and exposed to formaldehyde gas at various RH at 25°C.

| Relative Humidity | Per cent recovery at exposure time |
|-------------------|-----------------------------------|
|                   | 1 hr | 2 hr | 3 hr | 4 hr | 7 hr | 17 hr |
| 11 P              | 47.1 | 10.9 | 3.9  | 0.37 | 0.0035 | 0.0008 |
| 33 G              | 80.8 | 46.3 | 27.1 | 3.2  | 0.018  | 0.0016 |
| CP                | 62.9 | 20.4 | 7.0  | 0.6  | 0.0056 | 0.0003 |
| 53 P              | 35.4 | 2.6  | 0.34 | 0.024 | 0.00015 | 0 |
| 75 G              | 74.2 | 64.2 | 61.3 | 24.1 | 4.9    | 0.005  |
| CP                | 71.6 | 41.2 | 27.6 | 11.0 | 0.061  | 0 |
| 100 P             | 50.3 | 3.9  | 0.092| 0.008 | 0.0005 | 0 |
| G                 | 100  | 77.5 | 85.2 | 35.5 | 30.3   | 2.3    |
| CP                | 96.5 | 57.3 | 43.6 | 37.6 | 9.1    | 0 |
| 75 P              | 12.8 | 2.2  | 0.005| 0.0003| 0      | 0 |
| G                 | 99.0 | 64.7 | 34.6 | 40.3 | 2.9    | 0 |
| CP                | 82.9 | 76.3 | 48.9 | 47.7 | 9.8    | 0 |
| 100 P             | 52.3 | 4.2  | 0.69 | 0.15 | 0      | 0 |
| G                 | 72.8 | 27.7 | 6.6  | 2.0  | 0.0005 | 0 |
| CP                | 99.1 | 97.5 | 100  | 85.0 | 30.8   | 0.022 |

* Formaldehyde concn. = 3.5 mg HCHO/liter vaporized from paraformaldehyde.

* P, paper; G, glassine; CP, cotton plug.

**FIG. 3.** Death rate of B. subtilis var niger spores in glass tubes closed with various materials and exposed to formaldehyde from paraformaldehyde or Formalin at RH 33%.
**FIG. 4.** Death rate of *B. subtilis* var. niger spores in glass tubes closed with various materials and exposed to formaldehyde from paraformaldehyde or Formalin at RH 100%.

**FIG. 5.** Death rate of *B. subtilis* var. niger spores in glass tubes closed with paper and exposed to formaldehyde from paraformaldehyde at various RH levels.

**FIG. 6.** Death rate of *B. subtilis* var. niger spores in glass tubes closed with glassine and exposed to formaldehyde from paraformaldehyde at various RH levels.

**FIG. 7.** Death rate of *B. subtilis* var. niger spores in glass tubes closed with cotton plug and exposed to formaldehyde from paraformaldehyde at various RH levels.
heated. Vaporization required 1 to 2 min. The stop- 
cock was then closed, and the desiccator was stored at 
25°C for 1, 2, 3, 4, 7, or 17 hours. Only one exposure 
could be made in a desiccator at a time because, once 
the top was removed to retrieve the tubes within, the 
precise atmospheric condition and formaldehyde concen-
tration would be altered. Two theoretical form- 
aldehyde concentrations were used in this study, 3.5 
and 10.6 mg/liter.

To assay the paper patches, the tube closure ma-
terial was removed from the test tubes and the patch 
was transferred aseptically to a 10-ml sterile distilled-
water blank. The blank was shaken vigorously by hand 
until the paper patch disintegrated. Dilutions of 1:2, 
1:10, 1:100, and 1:1,000 were placed in petri dishes. 
Molten tryptose agar was added and mixed. All plates 
were incubated 3 to 5 days at 37°C before counting. 
Along with these, contaminated (control) patches not 
exposed to formaldehyde were also plated. All expo-
sures were repeated four times ranging from 1 to 4 
weeks apart. No inhibitory effect of the formaldehyde 
was found in these tests because of the dilution factor.

RESULTS AND DISCUSSION

Table 1 shows the sporicidal activity of the low 
ccentration (3.5 mg/liter) of formaldehyde 
eparized from paraformaldehyde. By comparing 
ese results with those shown graphically in 
Fig. 5, 6, and 7, it was determined that the higher 
formaldehyde concentration killed the B. subtilis 
spores at a significantly higher rate. Figure 3 
compares the activity of 3.5 mg of formaldehyde 
per liter generated from paraformaldehyde and 
also from Formalin at 33% RH. With each of 
the three closures, the Formalin-generated form-
aldehyde showed the slowest rate of kill. Part of 
the reason for this was that, after vaporizing, 
some of the formaldehyde and water condensed 
as small droplets on surfaces in the desiccator top 
that could not be heated, so that a lower concen-
tration of formaldehyde vapor was realized. In 
Fig. 4, no difference in rates of kill due to the 
two formaldehyde sources is observed for any 
one closure at 100% RH. Braswell and Hoffman 
in press) showed that the adsorption of form-
aldehyde from paraformaldehyde on material 
such as cloth increases with the RH as high as 
83%, but at 100% a lower adsorption results. 
At 100% RH, a greater quantity of water would 
be present on the desiccator surfaces to adsorb 
formaldehyde, thus lowering the vapor concen-
tration. It is suggested that the vapor concentra-
tions are comparable at 100% RH whether the 
formaldehyde was generated from Formalin or 
paraformaldehyde; consequently, there would 
be no difference in death rates. There is no reason 
to assume that formaldehyde generated from 
paraformaldehyde is any different from that 
generated from Formalin.

At the higher concentration of formaldehyde, 
i.e., 10.6 mg/liter (theoretical), a faster kill 
ocurred. Actually this is above saturation con-
centration, and, consequently, a white film of 
polymerized formaldehyde frequently appeared 
on the surfaces within the desiccator. The effect of 
RH on the penetrability of formaldehyde 
generated from paraformaldehyde at this 
concentration is shown in Fig. 5, 6, and 7. It was 
determined that there was no significant difference 
in death rate for the spores in a paper enclosure 
exposed to formaldehyde at RH of 33 to 100%, 
although at 11% RH the rate of kill was some-
what slower (Fig. 5). At the lower formaldehyde 
concentration (3.5 mg/liter), there was a definite 
effect of RH on the rate at which spores were 
killed. This suggests that high formaldehyde 
concentration helps to overcome the role of mois-
ture in bacterial spore death. Figure 6 shows the 
rate of formaldehyde penetration through glas-
sine paper. The interesting feature in these results 
is that glassine at intermediate humidities was 
slowly penetrated by the formaldehyde but at 
very high RH the penetration rate was signifi-
cantly faster, indicating that high moisture con-
tent probably affects the structure of glassine 
paper.

Figure 7 shows the effect of RH on the pen-
etration of cotton by formaldehyde. Here there 
was an opposite effect from glassine, in that at 
high RH the penetration rate was significantly 
lower than at lower RH. This is most likely due 
to a higher moisture content on the cotton 
surface that adsorbs and holds the formaldehyde, 
slowing its rate of penetration.

It is apparent that generalizations about the 
effect of humidity on the penetrability of form- 
aldehyde vapor are difficult to make because the 
effect varies with the nature of the material 
being penetrated. The results presented help to 
explain why previous investigators were not suc-
cessful in sterilizing microorganisms embedded 
in layers of cotton with formaldehyde at high 
RH. We believe that there is no difference in the 
activity of formaldehyde generated from Forma-
lin or paraformaldehyde if it is possible to pre-
vent recondensation of the Formalin after vapor-
ization, thus giving comparable vapor concen-
trations.

LITERATURE CITED

1. Committee on Formaldehyde Disinfection. 1958. Disinfection 
of fabrics with gaseous formaldehyde. J. Hyg. 56:488-515.
2. Phillips, C. R. 1968. Gaseous sterilization. In C. A. Lawrence 
and S. S. Black, (ed). Disinfection, sterilization, preservation. 
Lea and Febiger, Philadelphia.
3. Sykes, G. 1965. Disinfection and sterilization, 2nd ed. E. & 
F. N. Spon. LTD, London.