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Study of the effect of atmospheric relative humity (RH) on the adsorption of paraformaldehyde-generated formaldehyde gas on various surfaces and the effect of the adsorbed formaldehyde on the death rate of bacterial spores showed that increasing the RH caused a corresponding increase of formaldehyde levels on all surfaces. The amount peaked at 83% RH. The levels obtained at 100% RH were slightly below those at 83% RH. Cotton cloth had a much greater affinity for the gas at all RH than either glass or stainless steel. The death rate of bacterial spores on surfaces containing adsorbed formaldehyde was high for the first hour after removal from the formaldehyde atmosphere but decreased rapidly thereafter. This held true for both cotton and glass surfaces. Also, formaldehyde levels of 15 to 27 μg ml of nutrient broth caused inhibition of bacterial growth, but levels above 27 μg ml rendered broth sterile.

Formaldehyde has been used for years as a vapor-phase decontaminant to treat biologically contaminated enclosures. However, it has run into disfavor from time to time, primarily because of its ease of polymerization and the difficulty of polymer removal. In most of this work, Formalin was used as the source of formaldehyde. Both the inadequate method of dispensing the liquid and the lack of air circulation were undoubtedly the main causes of excessive polymerization. Because of its low vapor pressure, formaldehyde must be mixed rapidly with air to prevent condensation and polymerization. Recently, the thermal depolymerization of dry paraformaldehyde in small electric skillets has gained favor as a means of disseminating formaldehyde. This technique necessitates the use of many skillets when treating a large enclosure (2). Although the technique is very time-consuming and requires many electrical circuits, it does have the advantage of disseminating the chemical at many locations, giving a more uniform distribution and less condensation and polymerization in the enclosure. An unknown quantity in the use of formaldehyde for enclosure decontamination is the extent of chemical adsorption by various surfaces as a function of relative humidity (RH) and chemical concentration. The amount of formaldehyde adsorbed by a surface will affect the rate at which microorganisms are killed on that surface. Furthermore, the amount of adsorption will affect the kill rate of residual microorganisms after the surface is removed from the formaldehyde atmosphere.

The study reported here was initiated to (i) determine the levels of adsorbed formaldehyde acquired from vaporized paraformaldehyde and Formalin on several surfaces as a function of RH and chemical concentration, (ii) determine the level of formaldehyde required to inhibit the growth of *Bacillus subtilis* var. *niger* in broth, and (iii) determine the rate that microbial spores are killed on various surfaces by residual-adsorbed formaldehyde.

MATERIALS AND METHODS

The choice of test surfaces was based on the materials routinely used by our laboratory for bioindicators when decontaminating building interiors and in spacecraft sterilization studies.

Test A was performed to determine formaldehyde adsorption levels on cotton-cloth patches. Determinations were made after various formaldehyde concentrations, RH levels, and exposure times. Test B was performed to determine adsorption levels on stainless-steel surfaces. Test C investigated adsorption of formaldehyde on glass surfaces. Test D determined the levels of formaldehyde required to inhibit bacterial growth. Test E determined the rate at which bacterial spores were killed on cloth and glass surfaces by residual-adsorbed formaldehyde, and Test F compared the adsorption of paraformaldehyde and Formalin-generated formaldehyde on cotton-cloth patches.

Test A. Cotton cloth patches (5.8 inch diameter, ca. 1.59 cm, 0.06 g) were exposed to paraformaldehyde vapors (gaseous formaldehyde) in 9.4-liter desic-

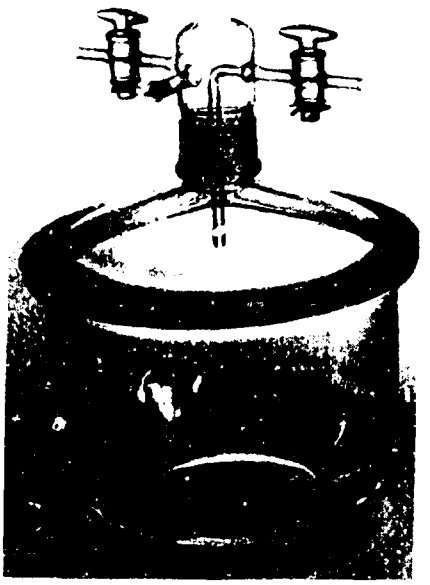

FIG. 1. *Formaldehyde exposure chamber.*

cators (Fig. 1). Each desiccator top was equipped with two stopcock-control ventilation tubes for purging the atmosphere with air of controlled RH and a ground-glass stoppered side arm to introduce and depolymerize the paraformaldehyde. All patches were conditioned for a minimum of 48 hr at the humidity level at which they were to be tested. Then, three patches per test were placed on the ends of pins set in metal plates on the bottom of the desiccator. Air with the proper RH was flushed through the desiccator through the ventilation tubes for a minimum of 15 min before each exposure. Paraformaldehyde was weighed into a small glass boat that fitted into the desiccator side arm. The side arm was then sealed with a ground glass stopper. To compensate for excess pressure during vaporization of the paraformaldehyde, a water-filled manometer tube was connected to the ventilation outlet; that stopcock remained open during the heating period. (This procedure prevented the dessicator lid from being blown off if the formaldehyde was accidently ignited through too vigorous heating of the side arm.) The paraformaldehyde was then vaporized by 1 to 2 min of gentle heating of the side arm with a bunsen burner. Timing of the exposure began after vaporization was completed. RH levels of 1.5, 50, 83, and 100%, and exposure times of 30, 60, and 120 min were used for each of the following paraformaldehyde concentrations: 10.6, 5.3, 2.7, 0.5, and 0.1 mg/liter. For each set of conditions, three patches were exposed and analyzed for formaldehyde.

The adsorbed formaldehyde was removed from the patches by vigorous agitation with 5 to 10 ml of hot distilled water. (The amount of water used was dependent upon expected concentration of the formaldehyde to get a satisfactory adsorption reading.) The formaldehyde content was analyzed by absorption spectroscopy with a Coleman Universal Spectrophotometer and phenylhydrazine hydrochloride-potassium ferricyanide color development (1). Absorption was read at a wavelength of 520 nm.

Test B. Small stainless steel plates (1 by 1 cm) were exposed to various concentrations of formaldehyde at different humidities (corresponding to test A). The exposure time was held constant at 60 min. Only one side of each plate was exposed; the other side was flat against the desiccator bottom. Formaldehyde was removed from the surface and analyzed in the same manner as in test A. When a determination showed no formaldehyde present, no lower concentrations or humidities were used.

Test C. Test C was performed in the same manner as test B, except that glass plates (1.4 by 1.4 cm) were used. The plates were cut from noncorrosive hard-glass microscope slides.

Test D. For determining the level of formaldehyde at which growth inhibition occurred, cotton patches with various levels of adsorbed formaldehyde were placed in sterile 10-ml nutrient broth blanks. The blanks were seeded with 10^5 *Bacillus subtilis* var. *niger* spores and incubated at 37 C. Periodic visual checks for cloudiness of the broth confirmed growth.

After incubation for 1 day, 1 ml from each blank showing no cloudiness was added to another 10 ml sterile broth blank. The initial portion of broth was identified as "A" broth, the diluted portion as "B" broth. Both samples were then returned to the incubator so that growth rates could be compared.

Test E. The effect of adsorbed formaldehyde on the rate of kill of *B. subtilis* var. *niger* spores was determined on cotton patches and glass squares. The square surfaces (0.5 by 0.5 inch, 1.27 by 1.27 cm) were contaminated, preconditioned to 53% RH, and then exposed to approximately 5 mg of formaldehyde (from paraformaldehyde) per liter at 53% RH and 25 C for 30 min. The selection of a short exposure was based on preliminary experiments, to give a reasonable time for formaldehyde adsorption by the surface yet result in only about 90% kill of the contaminating spores. At the end of the 30-min exposure, the surfaces were removed from the formaldehyde atmosphere placed in open petri dishes surrounded by wet towels to raise the RH to 50 to 60%, and allowed to aerate. After 0, 0.5, 1, 2, 4, 6 or 7, and 24 hr of aeration, the various materials were assayed for viable spores. Concurrently, surfaces were also assayed for adsorbed formaldehyde for comparison of formaldehyde concentration with death rate on each material.

Test F. The adsorption of formaldehyde generated from paraformaldehyde and from Formalin was compared under like conditions of RH and vapor concentration. Only cloth patches were used in this study.

RESULTS

All concentrations shown in the tables and graphs represent an average of three separate determinations.

Test A. As shown in Table 1, the higher the formaldehyde concentration at a given RH and exposure time, the more formaldehyde adsorbed

TABLE 1. *Amount of formaldehyde adsorbed on cotton patches from various amounts of vaporized paraformaldehyde*

Vapor concn (mg/liter)	Relative humidity	Formaldehyde (μg) adsorbed after exposure time of		
		30 min	60 min	120 min
0.12	1.5	4	5	6
	50	25	63	70
	83	73	94	215
0.53	1.5	14	14	16
	50	113	207	317
	83	250	473	552
2.7	1.5	27	31	42
	50	507	587	670
	83	1,394	1,758	1,650
	100	612	829	763
5.3	1.5	25	36	46
	50	607	993	1,150
	83	2,550	2,483	2,400
	100		1,250	
10.6	1.5	70	125	153
	50	1,267	1,430	1,783
	83	3,233	3,600	3,938
	100	783	2,091	2,616

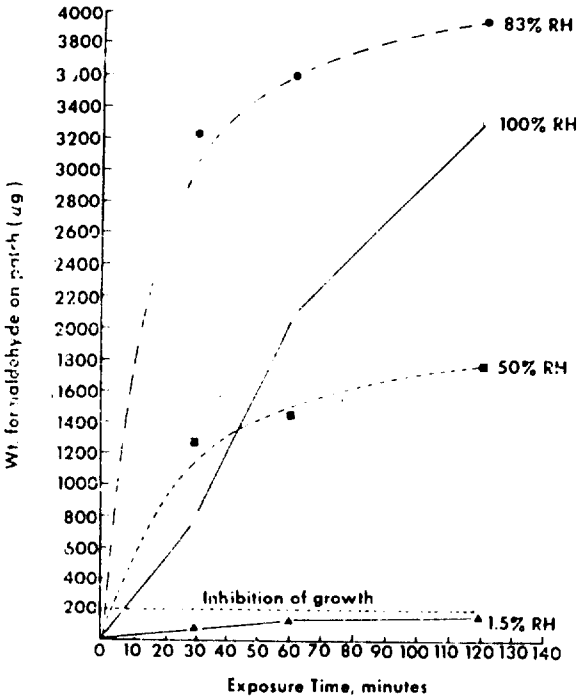

FIG. 2. *Amount of formaldehyde from paraformaldehyde adsorbed on cotton patches (5.8 inch, ca. 1.59 cm²). Vapor concentration is 10.6 mg/liter of air.*

TABLE 2. *Amount of formaldehyde adsorbed on stainless-steel plates in 60 min of exposure to various concentrations of vaporized paraformaldehyde*

Relative humidity	Paraformaldehyde concn (mg/liter)	Formaldehyde adsorbed (μg/cm²)
1.5	10.6	Trace
50	2.7	0
50	5.3	4
50	10.6	140
83	0.23	0
83	2.7	0
83	5.3	48
83	10.6	187
100	2.7	4
100	5.3	17

TABLE 3. *Amount of formaldehyde adsorbed on glass plates in 60 min of exposure to various concentrations of vaporized paraformaldehyde*

Relative humidity	Paraformaldehyde concn (mg/liter)	Formaldehyde adsorbed (μg/cm²)
1.5	2.7	0
1.5	5.3	0
1.5	10.6	Trace
50	0.23	0
50	2.7	2
50	5.3	32
50	10.6	182
83	0.23	0
83	2.7	0
83	5.3	45
83	10.6	217
100	0.23	0
100	2.7	20
100	5.3	64

on a patch. Also, the formaldehyde concentration on a patch increased with time (Table 1, Fig. 2), but the rate of adsorption was much higher the first hour of exposure and tended to decline rapidly after that period. About 80% of the 2-hr total was adsorbed by the end of the first hour.

Most profound was the effect of RH on the amount of formaldehyde adsorbed on a patch (Fig. 2). At RH levels as high as 83%, patch adsorption increased with increased humidity. Of the humidities tested, peak adsorption was reached at 83% RH. At 100% RH, formaldehyde levels fell between those at 50 and 83% RH, probably because more moisture at 100% RH enabled the desiccator surfaces to compete with the test surfaces for formaldehyde.

Test B. Stainless steel showed the least for

TABLE 4. *Inhibition in broth blanks*

Determination	*B. subtilis* growth at initial paraformaldehyde concn of (μg/ml of broth)											
	0	4	6	12	15[a]		23		27		32	
					A	B	A	B	A	B	A	B
Growth 1 day	+	+	+	+	−		−		−		−	
Growth 2 days					−	+	−	+	−	−	−	−
Growth 3 to 7 days					+		+		+	+	−	−
Reseeded at 7 days; growth within 2 days											+	+

[a] A = initial 10 ml of seeded broth; B = 1 ml of "A" diluted with 10 ml of fresh broth after "A" was incubated for 1 day.

maldehyde adsorption of the materials tested. Detectable amounts (at the humidities tested) were found only at 50% RH and above and then only at the higher formaldehyde concentrations. As with the cotton patches, maximum adsorption was at 83% RH. Table 2 gives the values obtained for stainless steel surfaces (1 by 1 cm).

Test C. The amount of formaldehyde adsorbed on the glass surfaces, although slightly higher than for stainless steel, was still of the same order of magnitude. Highest concentrations were again found at 83% RH. However, the formaldehyde levels were still far below those found on cotton patches. No formaldehyde was found below an RH of 50%. Under the conditions of 83 and 100% RH, at concentrations of paraformaldehyde of 5.3 mg/liter and above, formaldehyde appeared to be preferentially polymerized on the glass test surfaces. A thin white film appeared on the test surfaces that was not visible on the petri dish cover holding the samples nor on the interior surfaces of the desiccator. The sample surfaces were washed thoroughly with soap and hot water, and the exposures were repeated. The same results were observed, although not to the same extent as in the previous exposure. The formaldehyde concentrations reported in Table 3 are those found on the washed surfaces.

Test D. Cotton patches with formaldehyde concentrations of less than 15 μg ml of broth had no noticeable effect on *B. subtilis* var. *niger* growth in nutrient broth. Growth, as evidenced by cloudiness of the broth, was apparent after an incubation period of 24 hr. Patches with 15 to 27 μg of formaldehyde per ml of broth retarded growth for as long as 7 days. However, 1 ml taken from the 15- to 27-μg group and added to another 10-ml broth blank consistently showed growth sooner than the mother broth. Formaldehyde levels above 27 μg/ml rendered the broth sterile. These results are summarized in Table 4.

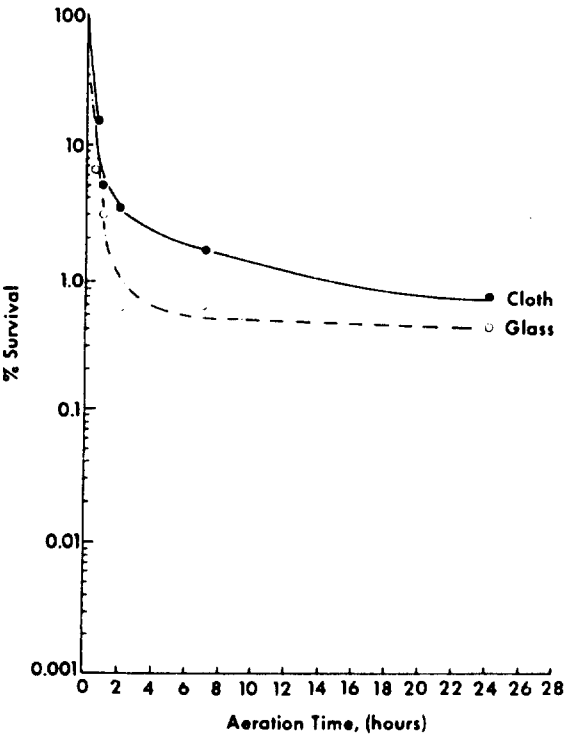

FIG. 3. *Residual activity of adsorbed formaldehyde against B. subtilis var. niger spores.*

Test E. The results of test E are shown graphically in Fig. 3. As expected, the spores continued to be killed for a time on both cloth and glass surfaces after being removed from the formaldehyde atmosphere. During the first hour of aeration, the death rate due to the adsorbed formaldehyde almost equaled that for spores in the formaldehyde vapor itself (where 84 to 93% were killed in the 30-min exposure). After 3 hr of aeration, however the death rate decreased rapidly; only a minimum of kill occurred thereafter. Although the data are not reported here, an additional test was performed to assure our-

TABLE 5. *Amount of formaldehyde adsorbed on cotton cloth patches exposed for 1 hr to vapor from paraformaldehyde and Formalin at 25 C*

Relative humidity	Amt of formaldehyde per desiccator (mg)	Avg adsorption per patch (μg)	
		Paraformaldehyde	Formalin
100	50	1,250	852
83	50	2,483	1,459
50	50	993	537

TABLE 6. *Desorption of formaldehyde from cloth patches and glass squares at 25 C*

Aeration time (hr)	Amt of HCHO per patch (μg)	
	Cloth	Glass
0	555	36
0.5	445	25
1	390	21
2	350	9
4	255	0
6	188	
24	24	

selves that the death of the cells was due to formaldehyde adsorbed on the cloth and glass surfaces and not just adsorbed on the bacterial spore surface. In this experiment, the cloth and glass patches were first exposed to the formaldehyde atmosphere for 30 min as before and then exposed to an aerosol of the bacterial spores for 15 min. The rate of kill of these spores on the formaldehyde-containing cloth and glass was in the same order of magnitude as in the above experiment. Table 6 shows desorption data generated from test E.

Test F. Table 5 shows the comparison of adsorbed formaldehyde concentrations on cloth patches when the formaldehyde was generated from paraformaldehyde or Formalin. At all three RH levels, less formaldehyde was adsorbed from vaporized Formalin. Actually, during the vaporization process, some liquid condensed into small droplets on desiccator surfaces that were not accessible to heating. It was found that 12% of the total formaldehyde vaporized was present in the condensed areas.

DISCUSSION

The results presented show that surfaces readily adsorb formaldehyde, the amount being dependent on the nature of the surface, the RH, and the exposure time. Such factors should be considered when establishing the protocol for decontaminating an enclosure and assessing the effectiveness of the procedure. It is evident that cloth is not a desirable surface for a biological indicator when evaluating the effectiveness of formaldehyde gas.

It is interesting that the death rate of microorganisms due to adsorbed formaldehyde is the same whether on glass or cloth, yet the amount of adsorbed formaldehyde is more than an order of magnitude different on the two surfaces. The sporicidal activity ceased after the concentration dropped from about 500 to about 300 μg on cloth and after dropping from 36 to less than 20 μg on glass. The vast difference in concentration may be associated with the difference in surface area of the two materials; therefore, at any particular isolated point the formaldehyde concentration could be the same regardless of surface. This would explain the similarity in death rates shown in Fig. 3 for glass and cloth surfaces.

In general, less formaldehyde was adsorbed on cloth when the chemical was generated from Formalin than from paraformaldehyde. The difference is probably entirely due to the condensation of some of the Formalin after vaporization.

ACKNOWLEDGMENT

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